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(54) Title: CHIMERAS OF HEPATITIS C VIRUS AND BOVINE VIRAL DIARRHEA VIRUS

(57) Abstract

Disclosed is a polynucleotide comprising a chimeric viral RNA which contains: a 5' nontranslated region (5' NTR), an open reading frame (ORF) region, and a 3' nontranslated region (3' NTR) wherein at least one of said regions is chimeric. The chimeric region comprises a first nucleotide sequence from a pestivirus in operable linkage with a heterologous nucleotide sequence. The chimeric viral RNA is replication-competent. Preferably the pestivirus sequence is from a bovine viral diarrhea virus and the heterologous nucleotide sequence is from a hepatitis C virus. Also disclosed are a method for identifying compounds having antiviral activity against hepatitis C virus, a genetically-engineered chimeric RNA virus and a vaccine against bovine viral diarrhea virus.

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Chimeras of Hepatitis C Virus and Bovine Viral Diarrhea VirusReference to Government Grant

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5

Related Applications

This application claims priority to, and incorporates herein in its entirety, U.S. 60/082,964 filed April 24, 1998.

10 Background of the Invention

## (1) Field of the Invention

This invention relates generally to the development of therapies for treating hepatitis C virus (HCV) and bovine viral diarrhea virus (BVDV) and more particularly to the identification of such therapies using chimeric viruses comprising a genomic sequence derived from HCV and bovine viral diarrhea virus (BVDV).

## 15 (2) Description of the Related Art

The *Flaviviridae* is an important family of human and animal RNA viral pathogens (Rice, CM. 1996. *Flaviviridae: The viruses and their replication*. In: Fields BN, Knipe DM, Howley PM., eds. *Fields virology*. Philadelphia: Lippincott-Raven Publishers. pp. 931-960.)

20 The three currently recognized genera of the *Flaviviridae* family exhibit distinct differences in transmission, host range, and pathogenesis. For example, members of the classical flavivirus genus, such as yellow fever virus and dengue virus, are typically transmitted to vertebrate hosts via arthropod vectors and cause acute self-limiting disease (Monath TP, Heinz FX. 1996. *Flaviviruses*. In: Fields BN, Knipe DM, Howley PM., eds. *Fields virology*. New York:

25 Raven Press. pp. 961-1034). The pestiviruses, such as bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV), cause economically important livestock disease and are spread by direct contact or the fecal-oral route (Thiel et al., 1996. *Pestiviruses*. In: Fields BN, Knipe DM, Howley PM., eds. *Fields virology*. New York: Raven Press. pp. 1059-1073). The most recently characterized *Flaviviridae* genus is the hepacivirus genus, the sole member 30 of which is the common and exclusively human pathogen, hepatitis C virus (HCV). HCV is

transmitted by contaminated blood or blood products and is the most common agent of non-A, non-B hepatitis, affecting more than 1% of the population worldwide (Houghton, 1996).

Hepatitis C viruses. In: Fields BN, Knipe DM, Howley PM., eds. *Fields virology*.

Philadelphia: Lippincott-Raven Publishers. pp. 1035-1058.). Unlike flavivirus and pestivirus

5 infections, which are usually eliminated by host immune response, chronic HCV infections are common and can cause mild to severe liver disease including cancer.

Despite these differences, members of the *Flaviviridae* family share common structural features and gene expression strategies. Virus particles consist of a lipid bilayer envelope with embedded transmembrane glycoproteins surrounding a protein-RNA

10 nucleocapsid. Genome RNAs are single-stranded of positive polarity, and function as the sole mRNA species for translation of a single long open reading frame (ORF). This ORF is translated into a polyprotein which is processed by cellular and viral proteases into mature viral proteins. Structural proteins destined for incorporation into virus particles are encoded in the N-terminal portion of the polyprotein, while the nonstructural proteins which form

15 components of the viral RNA replicase are encoded in the remainder.

Replication of the *Flaviviridae* RNA genome occurs via synthesis of a full-length negative-strand intermediate and is asymmetric, favoring synthesis of positive-strand RNAs.

However, little is known about the details of this process. For all three genera of the *Flaviviridae* family, full-length functional cDNA clones have been constructed and RNAs

20 transcribed from these cDNA templates are infectious. For flaviviruses and pestiviruses, mutagenesis of these clones and efficient RNA transfection of permissive cell cultures provides a means of probing the role of *cis* RNA elements and viral proteins in replicase assembly and function. Such analyses are not yet possible for HCV since this virus is unable to replicate efficiently in cell culture.

25 Like many other RNA viruses, it is believed the 5' and 3' terminal sequences of the *Flaviviridae* contain conserved *cis*-elements important for translation, RNA replication, and packaging (Bukh et al., *Proc. Natl. Acad. Sci. USA* 89:4942-4946, 1992; Deng et al., *Nucleic Acids Res.* 21:1949-1957, 1993; Cahour et al., *Virologist* 207:68-76, 1995; Kolykhalov et al., *J. Virol.* 70:3363-3371, 1996; Men et al., *J. Virol.* 70:3930-3937, 1996; Tanaka et al., *J. Virol.*

30 70:3307-3312, 1996; Huang HV. 1997. Evolution of the alphavirus promoter and the *cis*-acting sequences of RNA viruses. In: Saluzzo J-F, Dodet B. eds. *Factors in the emergence of arbovirus diseases*. Paris: Elsevier Press, pp. 65-79; Mandl et al., *J. Virol.* 72:2132-2140, 1998). The 5' nontranslated region (NTR) functions initially at the level of translation.

Similar to most cellular mRNAs, flavivirus genome RNAs are translated in a cap-dependent manner. These RNAs contain a 5' cap structure that is presumably added by virus-encoded

RNA triphosphatases, guanylyl-, and methyl-transferases (Rice, 1996, *supra*). In contrast, the translational strategy employed by pestiviruses and HCV is more similar to that of the picornaviruses. These RNAs appear to be uncapped and contain long 5' NTRs with *cis* RNA elements that function as internal ribosome entry sites (IRES) for translation initiation at the polyprotein AUG (Lemon et al., *Semin. Virol.* 8:274-288, 1997).

The 5' NTRs of HCV and BVDV have a similar structural and functional organization despite containing only short stretches of high sequence identity (Wang et al., *Curr. Top. Microbiol. Immunol.* 203:99-115, 1995; Lemon et al., 1997, *supra*). The IRES within each NTR is located at the 3' end of the NTR at a position proximal to the AUG initiation codon of the ORF. Although the 5' terminal sequence of each of these viruses is apparently not required for IRES function (Rijnbrand et al., *FEBS Lett* 365:115-119, 1995; Honda et al., *Virology* 222:31-42, 1996; Rijnbrand et al., *J. Virol.* 71:451-457, 1997), these sequences are highly conserved among different strains of HCV (Bukh et al., *Proc. Natl. Acad. Sci. USA*:89:4942-4946, 1992) or BVDV (Deng et al., 1993, *supra*), suggesting they play other roles in viral replication. For example, sequences in the 5' NTR may be required for regulating translation versus initiation of negative-strand RNA synthesis. Such regulation could occur by direct interaction of 5' and 3' RNA elements or indirectly, via RNA-protein interactions. Sequences in the 5' NTR may also modulate packaging versus translation. Finally, sequences complementary to the 5' NTR, which are located at the 3' end of negative-strand RNA, are likely to function in the initiation of positive-strand RNA synthesis.

The HCV 3' NTR contains an internal polypyrimidine tract followed by a highly conserved sequence of 98 bases at the 3' terminus, which has been shown to be required for replication of HCV (U.S. Application Serial No. 08/811,566).

Further elucidation of the role of sequences in the HCV 5' and 3' NTRs has been hampered by the inefficient replication of HCV in cell culture. This aspect of HCV biology also makes it difficult to identify and test possible antiviral compounds for activity against HCV. Thus, a need exists for a system which facilitates investigation of HCV replication and therapeutic approaches to control HCV infections.

30 Summary of the Invention

Briefly, therefore, the present invention provides novel compositions and methods for studying HCV replication which are based on the discovery that chimeras of HCV and BVDV genomic sequences can be constructed that are able to replicate in cell culture. The BVDV-specific sequence provides the chimeric viral nucleic acid with the ability to replicate in cell culture, while the HCV-specific sequence allows the chimeric viral nucleic acid to be used to

screen possible compounds for anti-viral activity against HCV. It is believed that similar replication-competent chimeras can be constructed from HCV and other pestiviruses.

Thus, in one embodiment, the present invention provides a novel, chimeric viral RNA in which at least one of the 5' NTR; ORF and 3' NTR regions is chimeric and comprises a nucleotide sequence from the corresponding region of a pestivirus in operable linkage with a nucleotide sequence from the corresponding region of an hepatitis C virus (HCV). The chimeric viral RNA is replication-competent. In preferred embodiments, the pestivirus is BVDV.

In other embodiments, the invention provides a polynucleotide comprising a DNA-dependent promoter operably linked to a cDNA of a chimeric viral RNA as described above and cells transiently transfected or stably transformed with the polynucleotide. In some embodiments the cDNA may encode a dominant selectable marker or an assayable reporter.

In yet another embodiment, the invention provides a method for identifying compounds having anti-HCV activity. The method comprises providing a first cell containing a chimeric viral nucleic acid derived from HCV and a pestivirus as described above and a second cell containing the pestivirus, and then comparing the replication efficiency of the chimeric viral nucleic acid in the presence and absence of a test compound to the replication efficiency of the pestivirus in the presence and absence of the test compound, wherein a greater reduction in compound-induced replication efficiency of the chimeric viral nucleic acid than the pestivirus indicates the compound has anti-HCV activity.

The invention also provides a genetically-engineered virus which comprises a chimeric viral nucleic acid derived from HCV and a pestivirus as described above. In one embodiment the genetically-engineered virus comprises virus particles containing at least one HCV structural protein and is useful in a vaccine against HCV. In another embodiment, the genetically-engineered virus is attenuated as compared to the pestivirus and is useful as a vaccine against the pestivirus.

In a still further embodiment, the invention provides a replication-competent BVDV vector expressing a heterologous sequence. The BVDV vector comprises the BVDV sequences encoding the BVDV replication machinery. In some embodiments, the replication-competent BVDV vector expresses an antigen and is useful as a vaccine.

#### Brief Description of the Drawings

Figure 1 is a schematic representation of the 5' NTRs of BVDV, HCV, and EMCV showing the position of the start codons of the ORF, and the boxes indicating the canonical IRES elements.

Figure 2 shows a schematic representation of BVDV and HCV chimeras, plaque phenotypes, reticulocyte translation efficiencies relative to parental BVDV, specific infectivities in MDBK cells, titers at 24 and 48 h post-transfection (or 72 h, as indicated), and an indication of whether pseudorevertants arose with results from BVDV, 5'HCV, BVDV+HCV, and BVDV+HCVdelB3 chimeras shown in Fig. 2A and results from BVDV+HCVdelB2B3, BVDV+HCVdelB1B2B3, BVDV+HCVdelB2B3H1, and BVDV+HCVdelB2B3H1H2 shown in Fig. 2B, where N.D. means not determined.

5 Figure 3 illustrates the *in vitro* translation efficiency of BVDV RNA or chimeras showing bar graphs of the amount of N<sup>pro</sup>, the N-terminal protein in the BVDV ORF, expressed by the various constructs.

10 Figure 4 illustrates a schematic representation of EMCV chimeras, plaque phenotypes, reticulocyte translation efficiencies relative to parental BVDV, specific infectivities in MDBK cells, titers at 24 and 48 h post-transfection (or 72 h, as indicated), and an indication of whether pseudorevertants arose.

15 Figure 5 illustrates a pseudorevertant analyses showing in (Fig. 5A) the relative positions of mutations detected within the plaque-purified variants of passaged BVDV+HCVdelB1B2B3, 5'EMCV, and 5'HCV, and in (Fig. 5B) the 5' terminal sequences of pseudorevertants of BVDV+HCVdelB1B2B3, 5'EMCV, and 5'HCV. Novel nucleotides or sequences are shown in bold upper case type. Pseudorevertants are numbered and designated by the suffix ".R". The upper case sequence in BVDV+HCVdelB1B2B3 and BVDV+HCVdelB1B2B3.R1 is a remnant of downstream BVDV 5' NTR sequences and was created during the cloning procedures.

20 Figure 6 illustrates the construction of derivatives of 5'HCV designed to contain 5' termini corresponding to the sequence detected within the three analyzed pseudorevertants. Fig. 6A shows the 5' terminal sequence of the 5'HCV derivatives with the suffix (orig) designating a derivative containing the original 5' terminal sequence of the pseudorevertant; the suffix (cons) designating a derivative containing the consensus tetranucleotide sequence 5'-GUAU at the same position; and novel sequences shown in bold upper case type. Fig. 6B shows plaque phenotypes, reticulocyte translation efficiencies relative to parental BVDV, specific infectivities in MDBK cells, and titers at 24 and 48 h post-transfection are indicated.

25 Figure 7 illustrates a single step growth curve for various chimeric constructs showing released virus titers measured by performing plaque assays on MDBK cells transfected with various constructs.

30 Figure 8 illustrates replication of BVDV RNA or chimeric derivatives in transfected MDBK cells. Equal numbers of MDBK cells (~ 8 x 10<sup>6</sup>) were electroporated with 5  $\mu$ g of

each *in vitro* synthesized RNA. MDBK cells were also transfected with infectious yellow fever 17D and Sindbis RNAs to provide molecular mass markers. One fifth of the transfected cells were seeded on 35-mm dishes and incubated in D-MEM supplemented with 10% horse serum for 6 h at 37°C. The media were then replaced with 1 ml of fresh media containing 2 g/ml of actinomycin D and 40 Ci/ml of <sup>3</sup>H-uridine. Incubations were continued for 10 h at 37°C. RNAs were isolated as described in Materials and Methods, and 1/4 of the samples was denatured in glyoxal and loaded on an agarose gel. (A) Autoradiograph of the dried gel. Only the portion of the gel containing the genomic RNAs is shown. (B) Amount of radioactivity contained within the displayed fragments as determined by scintillation counting. BVDV, lane 1; 5'HCV, lane 2; BVDV+HCVdelB2B3, lane 3; BVDV+HCVdelB2B3H1, lane 4; 5'HCV.R1orig, lane 5; 5'HCV.R1cons, lane 6; 5'HCV.R3orig, lane 7; 5'HCV.R3cons, lane 8; 5'HCV.R2orig, lane 9; 5'HCV.R2cons, lane 10; yellow fever 17D, lane 11; Sindbis, lane 12; non-transfected MDBK cells, lane 13. The experiments shown is one of two repetitions which yielded similar results.

Figure 9 illustrates the genetic map of plasmid pACNR/BUD.

Figure 10 illustrates the sequence of low copy number plasmid pACNR/BVDV NADL (circular) harboring the functional cDNA of cytopathic BVDV NADL (positive sense cDNA 5' to 3'; nt 1-12578).

Figure 11 illustrates the sequence of infectious BVDV NADL (positive sense cDNA 5' to 3').

Figure 12 illustrates the sequence of infectious non-cytopathic BVDV NADL lacking cIns (positive sense cDNA 5' to 3').

Figure 13 illustrates the sequence adapted HCV 5' NTR from 5'HCV/R1.cons (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 14 illustrates the sequence of adapted HCV 5' NTR from 5'HCV/R1.orig (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 15 illustrates the sequence of adapted HCV 5'NTR from 5'HCV/R2.cons (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 16 illustrates the sequence of adapted HCV 5' NTR from 5'HCV/R2.orig (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

Figure 17 illustrates the sequence of adapted HCV 5' NTR from 5'HCV/R3.cons (positive sense cDNA 5' to 3'; only the sequence from the 5'base to the ATG initiating the polyprotein is shown).

5 Figure 18 illustrates the sequence of adapted HCV 5'NTR from 5'HCV/R3.orig (positive sense cDNA 5' to 3'; only the sequence from the 5' base to the ATG initiating the polyprotein is shown).

10 Figure 19 illustrates the sequence of prototype HCV-BVDV chimera from pNADL/5'HR3.orig/3'H3'B with the adapted HCV 5'NTR from 5'HCV/R3.orig and tandem 3' NTR elements from HCV followed by BVDV (positive sense cDNA 5' to 3') as discussed in Example 5.

Figure 20 illustrates various deletions of the poly U track in the 3'NTR HCV sequence of BVDV/HCV chimera p5H-3H33.

Figure 21 illustrates the schematic representation of functional HCV-/BVDV chimera from pCBV/p7.

15 Figure 22 illustrates the sequence of functional HCV-BVDV chimera from pCBV/p7 (positive sense cDNA 5' to 3').

Figure 23 illustrates the schematic representation of a HCV/BVDV chimera with selectable marker.

20 Figure 24 illustrates the sequence of functional HCV-BVDV chimera from pCBV/p7/IRES-pac expressing a dominant selectable marker conferring resistance to puromycin (positive sense cDNA 5' to 3').

Figure 25 illustrates the schematic representation of a bicistronic HCV/BVDV chimera.

25 Figure 26 illustrates the sequence of functional bicistronic chimera expressing the entire HCV structural region derived from plasmid pNADL/BI#41/HCV str (positive sense cDNA 5' to 3')

#### Description of the Preferred Embodiments

In accordance with the present invention, the inventors herein have succeeded in generating HCV-BVDV chimeric RNAs which are replication competent. Such chimeras are useful in screening compounds *in vitro* for antiviral activity against HCV. In addition, it is believed that *in vivo* replication of HCV-BVDV chimeras according to the invention may be attenuated as compared to wild-type BVDV and thus may be useful in vaccinating animals against BVDV. It is also believed that the HCV chimeric structures described herein for BVDV are applicable to other pestiviruses.

In the context of this disclosure, the following terms will be defined as follows unless otherwise indicated:

- "Cis-acting sequences" means the nucleotide sequences from an RNA virus genome that are necessary for recognition of the genomic RNA by specific protein(s) of the RNA virus or host cell that carry out replication, transcription, translation or packaging of the genome.
- "Genetically-engineered virus" means any virus whose genome is different than that of a wild-type virus due to a human-made deletion, insertion, or substitution of one or more nucleotides to the wild-type viral genome.
- "Infectious" when used to describe a virus means the virus is capable of entering cells and initiating a virus replication cycle, whether or not this leads to the production of new RNA virus particles.
- "Nucleotide sequence" as used herein refers to DNA and the corresponding RNA sequence where relevant. It will be understood that sequences shown in the Figures are DNA versions of the RNA sequence and that chimeric molecules of the invention may comprise RNA molecules or cDNA copies of such RNA molecules.
- "Replication-competent" as applied to a chimeric HCV-pestivirus RNA means the RNA is capable of RNA-dependent replication in at least one cell type that supports replication of the wild-type parental pestivirus. The number of replicated RNA molecules produced by an HCV-pestivirus chimeric RNA of the invention is at least 10-fold higher than the limit of detection, which is typically 10 to 100 molecules. More preferably, chimeric RNA production by the HCV-pestivirus chimeric RNA is at least  $10^2$  to  $10^3$ -fold higher than the detection limit. The replication-competent chimeric RNA replicates at an efficiency that is preferably, at least 0.001%, more preferably, at least 0.01%, more preferably, at least 0.1%, more preferably, at least 1%, more preferably at least 10% and most preferably at least 50% up to 90% that of the parental pestivirus in the same cell type.
- "Transfected cell" means a cell containing an exogenously introduced nucleic acid molecule, and includes cells that are transiently transfected with the exogenous nucleic acid.
- "Transformed cell" or "stably transformed cell" means a cell containing an exogenously introduced nucleic acid molecule which is present in the cytoplasm or nucleus of the cell and may be stably integrated into the chromosomal DNA of the cell.
- "Virus" means a virion, virus particle or a viral genome.
- A chimeric viral RNA according to the invention is designed to comprise a 5' NTR, an ORF, and a 3' NTR, at least one of which is a chimeric region containing two operably linked nucleotide sequences that are from the same region of a pestivirus and an HCV.

Pestivirus-specific sequences useful in the invention can be taken from the appropriate genomic region of any cytopathic or noncytopathic type I or type II BVDV isolate, classical swine fever virus (CSFV) isolate, or border disease viral isolate. For a list of pestiviruses , see Thiel, H.-J., P. G. W. Plagemann, and V. Moennig. 1996. Pestiviruses, p. 1059-1073. In 5 B. N. Fields, D. M. Knipe and P. M. Howley (ed.), *Fields Virology*. Raven Press, New York. HCV-specific sequences can be taken from any strain or isolate of HCV, including but not limited to HCV-1, HCV-1a, HCV-1b, HCV-1c, HCV-2a, HCV-2b, HCV-2c, HCV-3a . Preferably, the parental pestivirus is a cytopathic strain of BVDV and the parental HCV strain is HCV-1.

10 The pestivirus- and HCV-specific sequences are operably linked in the chimeric region, meaning the sequences are arranged such that the resulting chimeric structure is functional in the context of replication of the pestivirus. For example, in one preferred embodiment the chimeric viral RNA comprises a chimeric 5' NTR which comprises a BVDV-specific 5' terminal sequence of 5'-(G/A)UAU and an IRES derived from HCV, with 15 the ORF and the 3' NTR consisting of a sequence from the same regions of BVDV. The BVDV-specific sequences at the 5' terminus and in the ORF and 3' NTR are chosen such that they are functional in the context of BVDV, meaning the chimeric viral RNA expresses the replication machinery of BVDV and this replication machinery is capable of replicating the chimeric RNA. In addition, translation of the BVDV ORF in the chimeric viral RNA is 20 dependent upon a functional HCV IRES. The presence of a functional HCV IRES in this chimera allows the chimera to be used to screen for compounds that target the HCV IRES and thereby inhibit translation of the BVDV ORF as well as replication of the chimeric virus. Such compounds would be expected to also inhibit translation of the ORF in a wild-type HCV and consequently inhibit HCV replication.

25 Compounds that could be screened for anti-HCV activity using this and other HCV-BVDV 5' NTR chimeras include but are not limited to antisense RNAs, RNA decoys that bind proteins involved in recognition of the HCV-specific sequences, ribozymes, and small molecule inhibitors of critical RNA-protein interactions. The use of such substances for therapeutic applications are known in the art. See, e.g., Amarzguioui M, et al., "Hammerhead 30 ribozyme design and application." *Cell Mol Life Sci.* 1998 Nov;54(11):1175-202; Welch PJ, et al., "Expression of ribozymes in gene transfer systems to modulate target RNA levels.", *Curr Opin Biotechnol.* 1998 Oct;9(5):486-96; Bramlage B, et al. "Designing ribozymes for the inhibition of gene expression."; *Trends Biotechnol.* 1998 Oct;16(10):434-8; Gewirtz AM, et al. "Nucleic acid therapeutics: state of the art and future prospects."; *Blood.* 1998 Aug 35 1;92(3):712-36; Altman S., "RNase P in research and therapy." *Biotechnology (N Y).* 1995

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It is contemplated that a number of replication-competent chimeric structures can be made that allow the function of various HCV sequence elements and proteins to be studied and targeted in drug screening assays. For example, the invention includes replication-competent HCV-pestivirus chimeras having a chimeric ORF. One such chimeric ORF is one 35 comprising an HCV sequence encoding the structural proteins and a pestivirus sequence

encoding the nonstructural proteins. It is believed that upon introduction into a cell, such a HCV-BVDV ORF chimera will produce HCV-like virus particles that will be released from the cell and capable of infecting cells normally infected by wild-type HCV, i.e., cells expressing an HCV receptor such as human CD81. Such ORF chimeras would be useful to screen compounds for drugs that inhibit formation, release or entry of HCV particles. In addition, ORF chimeras that produce virus particles containing at least one HCV structural protein would be useful as vaccines against HCV. Other ORF chimeras contemplated by the invention include, for example, chimeras comprising a pestivirus sequence encoding structural proteins and an HCV sequence encoding one or more nonstructural proteins such as the NS3 protease, NS4A cofactor, NS5A phosphoprotein/interferon resistance determinant and/or the NS5B polymerase. Replication of such ORF chimeras would be dependent upon the function of the HCV nonstructural protein(s) and these ORF chimeras could be used to screen for drugs that target the HCV nonstructural protein(s) as well as to screen for and map potential drug resistance mutations in HCV nonstructural proteins. In addition, HCV-pestivirus ORF chimeras could be useful for developing alternative *in vivo* animal models for HCV replication and HCV-associated hepatocellular carcinoma to evaluate antivirals and anti-tumor agents.

The invention also provides replication-competent HCV-pestivirus chimeras having a chimeric 3' NTR which contains one or more conserved elements of the HCV 3' NTR. Such 3' NTR chimeras would be useful for screening or evaluating compounds targeted against the HCV 3' NTR. Compounds that could be screened include antisense RNA molecules, ribozymes and small molecule inhibitors of critical RNA-protein interactions. One 3' NTR chimera according to the invention comprises a BVDV 5' NTR, BVDV ORF and a chimeric 3' NTR which consists of an HCV-specific sequence derived from the HCV 3' NTR immediately followed by a BVDV 3' NTR. The HCV-specific 3' NTR that allows for replication in the context of BVDV has a deletion in the 3' NTR poly (U) tract but has all the other HCV 3' NTR elements, including the 98 bp 3' terminal conserved element.

HCV-pestivirus chimeras included within the scope of the invention include those comprising combinations of chimeric regions, i.e., 5' NTR and ORF chimeras; 5' NTR and 3' NTR chimeras; ORF and 3' NTR chimeras; and chimeric RNAs in which each of the 5' NTR, ORF and 3' NTR regions comprise an HCV sequence operably linked to a pestivirus sequence.

The invention also provides chimeric RNAs having two ORFs, or bicistronic HCV-pestivirus chimeras. Bicistronic chimeras contemplated by the invention include structures in which the first ORF contains one or more HCV genes and is followed by a second IRES

operably linked to a second ORF encoding the pestivirus replicase machinery. It is also contemplated the first ORF may encode a heterologous sequence such as an antigen.

It is believed that many HCV-pestivirus chimeras of the invention will be attenuated as compared to the parental wild-type pestivirus. Such attenuated chimeric RNA genomes 5 would be candidate vaccines in the form of live-attenuated virus particles or as RNA or cDNA "genetic" vaccines.

The invention also includes vaccines against HCV which comprise an immunogenically-effective amount of HCV-pestivirus particles or nucleic acid. Anti-HCV 10 vaccines comprising virus particles should preferably contain one or more HCV structural proteins.

The therapeutic or pharmaceutical compositions of the present invention can be administered by any suitable route known in the art including for example by injection such as intraperitoneal, intravenous, subcutaneous, intramuscular, transdermal, intrathecal or 15 intracerebral injection. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation.

Compositions according to the invention can be employed in the form of pharmaceutical or veterinary preparations. Such preparations are made in a manner well known in the pharmaceutical and veterinary arts. One preferred preparation utilizes a vehicle 20 of physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers such as physiological concentrations of other non-toxic salts, five percent aqueous glucose solution, sterile water or the like may also be used. It may also be desirable that a suitable buffer be present in the composition. Such solutions can, if desired, be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for 25 ready injection. The primary solvent can be aqueous or alternatively non-aqueous.

The carrier can also contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate 30 of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or penetration across the blood-brain barrier. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dosage or multi-dose form or for direct infusion into the cerebrospinal fluid by continuous or periodic infusion.

It is also contemplated that certain formulations containing a chimeric virus according 35 to the invention are to be administered orally. Such formulations are preferably encapsulated and formulated with suitable carriers in solid dosage forms. Some examples of suitable

carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium, stearate, water, mineral oil, and the like. The 5 formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide rapid, sustained, or delayed release of the active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that diminish proteolytic degradation 10 and promote absorption such as, for example, surface active agents.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose will also be calculated dependent upon the particular route of administration selected. Such calculations can be made without undue experimentation by one skilled in the art. Exact dosages are 15 determined in conjunction with standard dose-response studies. It will be understood that the amount of the composition actually administered will be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration. Dose, 20 administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

Replication-competent HCV-pestiviruses are generated by choosing the HCV function or sequence element desired to be studied. The HCV sequence can be obtained from a plasmid clone of a partial or full HCV genome using PCR to amplify a target region 25 containing the desired sequence or by restriction enzyme digestion. The HCV fragment is then inserted into the desired location of a clone of the pestivirus genome using standard techniques. Desired portions of the pestivirus genome may be deleted before or after addition of the HCV fragment. The recombinant genome is then transfected into a cell that supports replication of the parental pestivirus genome and their ability to replicate using standard assays. For example, replication can be assessed by virus-induced cytopathic effect; plaque 30 formation; detection of viral antigens and/or viral RNA accumulation; and by plaque assay measuring released infectious virus. The inventors herein have found that the BVDV RNA replication machinery works in many cell types, including bovine, hamster, mouse and human cells. It has also been reported that BVDV RNAs can amplify in other cell types including 35 human hepatoma lines and hepatocytes (Behrens SE, et al., *J Virol.* 1998 Mar;72(3):2364-72).

The host cell range for a particular chimera will be dependent upon the properties of that chimera as empirically determined.

As described below, some chimeras do not replicate stably as indicated by heterogeneity in the size of plaques produced by the chimeric virus. Upon passage, pseudorevertants can frequently be isolated that are capable of stable replication. Such pseudorevertants will have one or more deletions or base substitutions in the HCV and/or pestivirus sequences. Information derived from these gain-of-function mutations can be used to define the elements necessary for generating stable, replication-competent chimeras of HCV and a pestivirus.

The invention provides a method for screening compounds for antiviral activity against HCV. The method involves comparing a test compound's effect on replication of a chimeric HCV-pestivirus RNA molecule as described above with the compound's effect on replication of the parental pestivirus. Compounds which have a greater effect on replication of the chimeric virus than the pestivirus are likely directed against the HCV portion of the chimera. Typically, the method is performed by providing duplicate cell cultures containing a chimeric viral RNA which is replication-competent in that cell, treating one of the culture with the test compound, and then measuring the replication efficiency of the chimeric RNA in both cultures. Any effect induced by the compound is compared against the compound's effect on replication of the parental pestivirus in cells of the same type. This control assay is preferably performed at the same time using the same culture conditions.

The cells used in the screening assay can be prepared by transiently transfecting the cells with the desired chimeric RNA molecule as described below. Alternatively, it is contemplated that the chimeric RNA molecule can be constitutively expressed in the cell by transfecting the cell with a polynucleotide comprising a cDNA of the chimeric RNA operably linked to a DNA-dependent promoter. The chimeric cDNA may include a selectable marker, which would allow for selection of cells expressing the chimeric RNA. It is also envisioned the selectable marker could be a dominant marker that allows selection of cells expressing chimeras having adaptive mutations or selection of cells permissive for virus replication (Frolov et al., *J. Virol.* 73:3854-3865, 1999). It is also contemplated the cDNA could express a reporter gene that could be assayed to measure RNA replication.

Alternatively, chimeric virus particles are incubated with a cell permissive for infection by the pestivirus in the presence or absence of the test compound and then replication of the chimeric virus is measured and compared to the replication of the parental pestivirus incubated with the same cell type in the presence or absence of the test compound.

Inhibition of replication can be measured in many ways, including assaying for the reduction of virus-induced cytopathic effect; inhibition of plaque formation, reduced production of viral antigens as detected by immunofluorescence assay; reduced viral RNA accumulation; reduction in released infectious virus from treated and untreated control and 5 chimera samples using a plaque assay. In addition, it is contemplated that a cell line that is designed for pestivirus-specific transactivation of a reporter gene could be used directly or in lieu of a plaque assay. The reporter gene is operably linked to a promoter that is activated upon infection by the chimeric virus and production of the viral transactivator protein.

Preferred embodiments of the invention are described in the following examples.

10 Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

15 **Example 1**

This example illustrates the construction and analysis of 5' HCV-BVDV chimeras as reported in detail in Frolov et al. (*RNA* 4:1418-1435, 1998) which is incorporated in its entirety by reference. A functional clone of BVDV (Mendez et al., *J. Virol.* 72:4737-4745, 1998) was used to construct and characterize a series of 5' NTR chimeras with sequences derived from HCV and the picornavirus, encephalomyocarditis virus (EMCV). The results 20 help to define the requirements of a functional BVDV 5' NTR and provide replication-competent BVDV-HCV chimeras dependent on a functional HCV IRES.

25 **Example 2**

This example illustrates the construction of chimeras for expressing additional functional portions of the HCV genome by addition of further HCV sequence downstream from the functional or adapted HCV 5'NTR chimeras fused in-frame to the BVDV ORF.

One such construct (Figure 21) involves fusion of HCV sequences to BVDV 30 sequences in the p7 protein coding region (at a convenient BseRI restriction site). Both HCV and BVDV encode a p7 protein that is located immediately downstream of the E2 protein. The p7 protein is a small hydrophobic protein of unknown function. pCBV/p7 consists of the first 79 bases of the BVDV 5'NTR encoding stem loop structure B1' and B1, followed by the entire HCV 5'NTR, the entire HCV structural protein coding region and the first 36 amino acids of HCV p7 fused to the C-terminal 31 amino acids of BVDV p7. The fused p7 gene is followed by the remainder of the BVDV ORF including the entire nonstructural region and 35 the BVDV 3' NTR. Transfection of MDBK cells with the RNA corresponding to this

sequence (Figure 22) leads to replication of the chimeric RNA and production of the expected HCV and BVDV polyprotein cleavage products. Variations on this strategy are envisioned in which all or part of the HCV polyprotein and cis elements important for RNA packaging can be expressed in viable chimeras. In addition the BVDV replicase regions for either cytopathic  
5 or non-cytopathic pestiviruses (like NADL cIns-) can be used. Transfection of cells permissive for HCV particle, assembly, release and reinfection with this chimeric RNA can be used to make HCV-like particles. These particles and this infection system can be used (i) to screen for specific inhibitors of HCV particle, assembly, release and reinfection, (ii) for identifying antibodies capable of neutralizing HCV infectivity and (iii) as live or inactivated  
10 vaccines. Furthermore, this embodiment of the invention demonstrates that the BVDV RNA replication machinery can be used for expression of heterologous RNA and polypeptide sequences and can be used as a vehicle for RNA or DNA "genetic" vaccination in which the BVDV replicase amplifies the level of antigen expression by cytoplasmic RNA-dependent replication.

15

### Example 3

This example illustrates chimeric RNA's that are modified to express dominant selectable markers, assayable markers or FACS sortable markers.

Such variants can be used to select for chimeras capable of replication in particular  
20 cell types, or to screen for cell types that are permissive for replication of the chimeric RNA. Selectable markers include, but are not limited to, the genes encoding puromycin resistance (puromycin N-acetyl transferase; PAC), neomycin resistance, blasticidin resistance, hygromycin resistance, etc. Assayable markers include, but are not limited to, the genes encoding B-galactosidase, luciferase, B-glucuronidase, etc. Easily sortable molecules include  
25 single chain antibodies, cell surface markers, and non-toxic protein markers like green fluorescent protein. In a specific example (Figures 23 and 24), the RNA encoded by pCBV/p7 was modified to include a cassette at the beginning of the BVDV 3'NTR that is comprised of the EMCV IRES driving the gene encoding PAC. This chimeric RNA can replicate, expresses PAC and confers resistance to puromycin resistance. This property can  
30 be used to select for variants of the chimera that are capable of noncytopathic replication in desired cells type and also provides a means of showing that cells harbor a functional chimeric RNA. Desired variants can be identified, cloned and further characterized as described in Example 1. Of note, is that this location in the BVDV genome and this strategy for expressing heterologous genes may also be applied to using infectious attenuated

pestiviruses as gene expression vectors and as chimeric live vaccines against other animal pathogens.

#### Example 4

5

This example illustrates the use of the bicistronic strategy as an alternative to the in-frame fusions described in Example 2.

A specific example is shown in Figure 25 and its sequence as Figure 26. In this bicistronic chimera, the 5' sequences are identical to that of pCBV/p7 except that the HCV 10 ORF continues to include the first 246 amino acids of NS4B. The HCV sequence is followed by the EMCV IRES fused to BVDV Npro, the N-terminal 10 aa of BVDV C, the C-terminal 19 aa of C, 9 N-terminal amino acids of Erns, 48 C-terminal amino acids of E2 and the remainder of the BVDV NADL ORF and 3' NTR. The constructed BVDV ORF encodes a functional BVDV RNA replicase. The deletions in the N-terminal portion of this ORF were 15 designed to preserve proper membrane topology and processing of the replicase. The bicistronic chimeric RNA can replicate upon transfection of permissive BVDV host cells.

#### Example 5

20 This example illustrates 3'NTR chimeras. Although initial attempts to recover viable chimeric viruses in which the BVDV 3'NTR was completely replaced by that of HCV were unsuccessful, a strategy similar to that detailed in Example 1 has produced chimeras that harbor the conserved elements of the HCV 3'NTR. An initial tandem 3'NTR construct was made in which the HCV 3'NTR was engineered to follow the BVDV ORF. The complete 25 BVDV 3'NTR was positioned 3' to the HCV 3' NTR after a short heterologous sequence. This sequence of this parental construct, which replicated poorly, is shown in Figure 19. RNAs transcribed from this plasmid were of low specific infectivity suggesting that revertants or pseudorevertants might have arisen. Indeed isolation and sequence analysis of several independent plaque-forming variants revealed that deletions in the HCV poly U tract of 30 various lengths had occurred. These revertant sequences are shown in Figure 20. When these altered HCV 3'NTRs were reconstituted into the original tandem 3' NTR parent, they gave rise to plaque forming RNA transcripts of high specific infectivity, demonstrating that these alterations restored the ability of the chimeric RNA to replicate. Large deletions in the U tract gave rise to virus with more robust replication and larger plaques while stably maintaining the 35 conserved HCV 3'NTR 98-base element and the polypyrimidine "transition" region. Such

chimeric viruses can now be used to screen and evaluate antisense, ribozyme, and other therapeutics targeted against this conserved HCV RNA element that is essential for replication.

5

## Materials and Methods

### Plasmid Constructs

pACNR/BVDV NADL was previously described (Mendez et al., 1998, *supra*). pBVDV is a derivative of pACNR/BVDV NADL which contains a G→T transversion at nt 14994 that creates an *Xba* I site upstream of the T7 promoter (T. Myers & C.M. Rice, unpubl.). To facilitate construction of the chimeras, subclones were created. First, two fragments were isolated by PCR amplification of p90/HCVFLlongpU (Kolykhalov et al., *Science* 277:570-574, 1997) with primers #498 (5'-TGTACATGGCACGTGCCAGCCCC) and #498 (5'-GATCAACTCCATGGTGCACGGTCT) and pBVDV with primers #481 (5'-AGACCGTGACCATGGAGTTGATC) and #482 (5'-CGTTTCACACATGGATCCCTCCTC). These two fragments were digested with *Apal* I and ligated to produce a fragment containing a fusion of the HCV 5' NTR to the BVDV ORF. This fragment was digested with *SacI* and ligated into pGEM3Zf(-) which had been digested with *Sma* I and *Sac* I to produce the subclone pGEM498-SacI. Next, a fragment containing the BVDV 5' NTR was synthesized by PCR amplification of pBVDV with primers #183 (5'-TTTCTAGATAATACGACTCACTATAGTATACGAGAATTAGAAAAGGCACTCG) and #480 (5'-GGGGGCTGGCACGTGCCATGTACA). This fragment was digested with *Xba* I and *BsrG* I and ligated into pGEM498-SacI digested with the same two enzymes, to create the plasmid pGEMXbal-SacI. pGEMXbal-SacI contains a tandem fusion of the BVDV 5' NTR, the HCV 5' NTR, and the 5' portion of the BVDV N<sup>pro</sup> gene. pBVDV + HCV was created by digesting pGEMXbal-SacI with *Xba* I and *Sac* I and ligating the fragment into pBVDV digested with the same two enzymes, and as such pBVDV + HCV contains the T7 promoter, followed by the entire 385-nt 5' NTR of BVDV, a GT dinucleotide (nt 386-387), the entire 341-nt 5' NTR of HCV (nt 388-728), and the sequence of the BVDV NADL strain including the ORF and 3' NTR. Derivatives of pBVDV + HCV containing deletions within the BVDV 5' NTR and/or the HCV 5' NTR were created in the subclone pGEMXbal-SacI, as described below, prior to ligation into *Sba* I- and *Sac* I-digested pBVDV. For making deletions, restriction sites with non-compatible protruding ends were treated with the Klenow fragment of DNA polymerase I prior to ligation. For creation of pBVDV + HCVdelB3 (deletion of nt 174-374, inclusive), pGEMXbal-SacI was digested with *Afl* II and *BsrG* I. For pBVDV + HCVdelB2B3 (deletion of nt 67-374), pGEMXbal-SacI was digested

with *Avr* II and *BsrG* I. For pBVDV + HCVdelB1B2B3 (deletion of nt 33-374), pGEMXbal-SacI was digested with *SnaB* I and *BsrG* I. For pBVDV + HCVdelB2B3H1 (deletion of nt 67-3396), pGEMXbal-SacI was digested with *Avr* II and *Xcm* I. For pBVDV + HCVdelB2B3H1H2 (deletion of nt 67-513), pGEMXbal-SacI was digested with *AVR* II and *Bsg* I. For pBVDV + HCVdelB2B3H3 (deletion of nt 67-374, 518-704), subclone pGEMXbal-SacI delB2B3 was digested with *Sma* I. p5'HCV was created by digesting p90/HCVlongpU with *Xba* I and *Nru* I and ligating the fragment into pBVDV + HCV digested with the same two enzymes.

The EMCV plasmid, pEC<sub>g</sub>, was provided by Ann Palmenberg and is described elsewhere (Hahn et al., *J. Virol* 69:2697-2699, 1995). p5'EMCV contains the entire 710 nt of the 5' NTR of EMCV, followed by the open reading frame of BVDV and the 3' NTR. One extra G residue was added between the T7 promoter and the first nucleotide of the EMCV 5' NTR to facilitate efficient in vitro transcription. Convenient restriction sites within the BVDV 5' NTR or the EMCV 5' NTR were used to create additional chimeras. Sites with noncompatible protruding ends were treated with the Klenow fragment of DNA polymerase I prior to ligation. For example, the plasmid pBVDV + EMCVdelA contains nt 1-378 of BVDV 5' NTR fused with nt 45-710 of EMCV (the *BsrG* I site of BVDV ligated to the *EcoR* V site of EMCV), pBVDV + EMCVdelB3A contains nt 1-173 of BVDV fused with nt 45-710 of EMCV (the *Afl* II site of BVDV ligated to the *EcoR* V site of EMCV). pBVDV + EMCVdelB2B3A contains nt 1-66 of BVDV fused with nt 45-710 of EMCV (the *Avr* II site of BVDV ligated to the *EcoR* V site of EMCV). pBVDV + EMCVdelB3ABC contains nt 1-173 of BVDV fused with nt 161-710 of EMCV (the *Afl* II site of BVDV ligated to the *Psp*1405 site of EMCV). pBVDV + EMCVdelB2B3ABC contains nt 1-66 of BVDV fused with nt 161-710 of EMCV (the *Avr* II site of BVDV ligated to the *Psp*1406 site of EMCV). pBVDV + EMCVdelB3A-H contains nt 1-101 of BVDV fused with nt 289-710 of EMCV (the *Nhe* I site of BVDV ligated to the *Avr* II site of EMCV). pBVDV + EMCVdelB2B3A-H contains nt 1-62 of BVDV fused with nt 289-710 of EMCV (the *Avr* II site of BVDV ligated to the *Avr* II site of EMCV). The schematics of the chimeric 5' NTRs are presented in Figures 2 and 4.

All other heterologous 5' NTRs used in the study were generated by PCR using an oligonucleotide complementary to nt 256-272 of the HCV 5' NTR and primers containing the sequence of the *Xba* I restriction site followed by the T7 promoter, the heterologous sequences found in sequenced pseudorevertants, or sequences corresponding to different regions of the HCV 5' NTR. All the fragments were subcloned into the plasmid, pRS2 (a derivative of pUC19), sequenced, and recloned into the p5'HCV plasmid by replacing the

fragment between the *Xba* I site located upstream of the T7 promoter and the *Nhe* I site (nt 249-254) in the 5' NTR of HCV.

#### Cell cultures

MDBK cells were obtained from M. Collett (ViroPharma, Inc.) and BT cells were 5 obtained from the American Type Culture Collection (Rockville, Maryland). Cells were grown in Dulbecco's modified Eagle medium (D-MEM) supplemented with 10% horse serum and sodium pyruvate.

#### Transcriptions and transfections

All the designed plasmids , including pBVDV and the chimeric derivatives, were 10 digested to completion with *Sda* I (Sse83871), purified by phenol extraction, precipitated by ethanol, and dissolved in water. The transcription reactions were performed sin the T7 Megascript kit (AMBION) using the conditions recommended by the manufacturer.

Reactions were incubated at 37°C for 1 h, and <sup>3</sup>H-UTP was added to the reaction to quantify 15 the RNA synthesis. The quality of the synthesized RNAs was checked by agarose gel electrophoresis, and samples containing 50-60% of full-length RNA were used for electroporations and in vitro translations. The reaction mixtures were aliquoted and stored at -70°C prior to electroporation or in vitro translations.

Transfection was performed by electroporation of MDBK cells using previously 20 described conditions (Mendez et al., 1998, *supra*). Two micrograms of in vitro synthesized RNA, corresponding to approximately 1 μ g of the full-length transcript, were used per electroporation. In standard experiments, ten-fold dilutions of electroporated cells were seeded in 6-well tissue culture plates containing  $5 \times 10^5$  naive MDBK cells per well. After 1 h of incubation at 37°C in an 5% CO<sub>2</sub> incubator, cells were overlaid with 3 ml of 0.6% LE Sea Kem agarose (FMC Bioproducts) containing minimal essential medium supplemented 25 with 5% horse serum. Plaques were stained with crystal violet after 3 days incubation at 37°C. The rest of the transfected cells was seeded into 100-mm dishes and incubated for approximately 48 h or until cytopathic effect was observed in virtually all cells. Samples of the media were taken at 24 and 48 h, and virus titers were determined as described above and previously (Mendez et al., 1998, *supra*).

#### Analysis of the 5' ends of viral genomes

Sequencing of the 5' ends of selected variants of BVDV was performed on plaque-purified viruses. Plaques were typically isolated from the agarose overlay without staining with neutral red. Virus was eluted in 1 ml of D-MEM/10% horse serum for several hours and was used to infect  $5 \times 10^5$  MDBK cells in 35-mm dishes. After 1 h of virus adsorption of 37

°C, an additional 1 ml of D-MEM/10% horse serum was added to the dishes, and incubation was continued for 36-48 h until cytopathic effect was observed in virtually all cells.

Fifty microliters of harvested viral stocks were clarified by low speed centrifugation, and viral RNAs were isolated by TRIzol reagent (Gibco-BRL) using the protocol recommended by the manufacturer. Sequencing of the 5' termini was performed using an oligonucleotide/cDNA-ligation strategy described elsewhere (Troutt et al., *Proc. Natl. Acad. Sci. USA* 89:9823-9825, 1992). The primer S1 (5'-GTCGTTCACACATGGATCC), complementary to nt 710-729 of the BVDV genome, was used for cDNA synthesis. A phosphorylated oligonucleotide tag (5'-GACTGTTGCGCTGCAGGGCCGAATT) with an amino group on the 3' terminus was ligated to the first strand cDNA (Troutt et al., 1992, *supra*). One tenth of this reaction mixture was used for PCR amplification. The primers for PCR amplification were as follows: primer A (5'-GCCCTGCAGGCCACAACAGTC), complementary to the tag; primer B (5'-TCAGGCAGTACCACAA) complementary to nt 281-296 of the HCV 5' NTR; and primer C (5'-GGAATGCTCGTCAAGAAGACAG), complementary to nt 268-289 of the EMCV 5' NTR. The primer pairs of A + B or A + C were used for analysis of the pseudorevertants of 5'HCV and BVDV + HCVdelB1B2B3 or 5'EMCV, respectively. For the 5'HCV pseudorevertants, one tenth of the ligation mixture was used for an additional PCR reaction. This fragment was synthesized using primer S1, describe above, and a primer corresponding to nt 147-175 of the HCV genome. Fragments were purified by agarose gel electrophoresis and cloned into the plasmid pRS2. Multiple independent clones were sequenced by the standard dideoxy-mediated chain termination methods using the Sequenase version 2.0 DNA Sequencing Kit (USB).

#### Cell-free translation

Cell-free translation reactions were performed in reticulocyte extracts (Promega) using conditions recommended by the manufacture. Usually 0.1-1 µg of the same in vitro synthesized RNAs used in transfection experiments were used in 25 µl translation reactions. After 45 min of incubation at 30 °C, 2 µl were dissolved in 10 µl of sample buffer, and those samples were analyzed by sodium dodecyl sulfate PAGE. Labeled proteins were visualized by autoradiography of the dried gel. The efficiency of translation was measured using phosphorimager analysis (Molecular Dynamics) by comparing the radioactivity in the band corresponding to the N<sup>Pro</sup> protein. In preliminary experiments, an eightfold increase in incorporation was observed for translation of 4 µg versus 0.4 µg BVDV transcript RNA. Quantitative data were obtained from reactions using subsaturating (0.4 µg) amounts of BVDV or BVDV chimera transcript RNAs.

### Analysis of virus specific RNAs

The protocols used for radioactive labeling of virus-specific RNAs are described in the appropriate figure legends. RNAs were isolated from the cells by using TRIzol reagent as recommended by the manufacturer (Gibco-BRL). After denaturation with glyoxal in dimethylsulfoxide, cellular RNAs were analyzed by electrophoresis in a 1% agarose gel containing a 10 mM phosphate buffer. Pieces of the dried gel containing the appropriate RNA bands were excised, and their radioactivity measured by liquid scintillation counting.

### **Results**

#### **10 Features of the BVDV, HCV, and EMCV 5' NTRs important for chimera design**

Schematic representations of the proposed secondary structures of the 5' NTRs of HCV, BVDV, and EMCV are shown, and the location of each IRES is indicated in Figure 1. EMCV is a member of the cardiovirus genus within the family *Picornaviridae*. While not a member of the *Flaviviridae*, EMCV is similar to HCV and BVDV in that it is a positive-strand RNA virus shown to contain an IRES within its 5' NTR (Jang et al., *J. virol.* 62:2636-2643, 1988). Based on their proposed secondary structures, the HCV IRES and the BVDV IRES have been classified as type 3 IRESs, while the EMCV IRES is classified as a type 2 IRES (Lemon & Honda, *Siemin. Virol.* 8:274-288, 1997). However, these three IRESs as well as IRESs from other members of the *Flaviviridae* and the *Picornaviridae* have been proposed to contain a common structural core (Le et al., *Virus Genes* 12:135-147, 1996).

The model for the secondary structure of the 341-nt HCV 5' NTR has been refined by enzymatic and chemical analysis of synthetic transcripts (Brown et al., *Nucl. Acids. Res.* 20:5041-5045, 1992; Wang et al., *J. Virol.* 68:7301-7307, 1994; Honda et al., *RNA* 2:955-968, 1996; Lima et al., 1997). This element contains four discreet hairpins (referred to here as H1, H2, H3 and H4) and a pseudoknot at the base of hairpin H3 (Wang et al., 1995). The secondary structure of the 385-nt BVDV 5' NTR has not been as extensively studied, but is proposed to be similar to that of HCV (Brown et al., 1992) with four discrete hairpins (referred to here as B1', B1, B2, and B3) and a pseudoknot at the base of B3 (Rijnbrand et al., 1997). The secondary structure of the longer (>700 nt) EMCV 5' NTR consists of a series of hairpins A-M (Duke et al., 1992; Hoffman & Palmenberg, 1996). Recently, a revised model of the EMCV 5' NTR suggests moderately different secondary structures for the C and G subregions, and significantly different secondary structures for the I-M subregion (Palmenberg & Sgro, 1997).

For HCV, H1 is nonessential for IRES function (Reynolds et al., 1995; Rijnbrand et al., 1995; Honda et al., 1996b; Reynolds et al., 1996; Kamoshita et al., 1997) and its deletion

has actually increased translation efficiency in some analyses (Rijnbrand et al., 1995; Honda et al., 1996b). Most studies have found that hairpin H2 and H3 and the pseudoknot are essential for IRES function (Wang et al., 1993; Rijnbrand et al., 1995; Honda et al., 1996b). However, two studies indicate that H2 may not be essential (Tsukiyama-Kohara et al., 1992; Urabe et al., 1997). The 3' boundary of the HCV IRES is more controversial. The IRES clearly extends to the AUG initiation codon. However, some studies indicate that sequences affecting the efficiency of translation initiation extend into the ORF (Reynolds et al., 1995; Honda et al., 1996a; Honda et al., 1996b; Lu & Wimmer, 1996). By analogy to the HCV IRES and the related pestivirus CSFV IRES, the BVDV IRES probably requires hairpins B2 and B3 and the pseudoknot for function, with B1' and B1 probably not required for IRES activity (Poole et al., 1995; Rijnbrand et al., 1997). For EMCV, hairpins H-L have been shown to be required for IRES function in mono- or dicistronic constructs (Jang & Wimmer, 1990; Duke et al., 1992). The remaining portion of the EMCV 5' NTR is thought to be required for RNA replication or unknown steps in viral replication that are important for pathogenesis (Duke et al., 1990; Martin & Palmenberg, 1996).

**Replacement of the BVDV 5' NTR with the HCV 5' NTR results in a large decrease in specific infectivity**

Since the BVDV 5' NTR and the HCV 5' NTR are proposed to have similar RNA secondary structure and functional organization, an experiment was performed to test whether the BVDV 5' NTR could be replaced by the HCV 5' NTR. p5'HCV has an exact replacement of the BVDV 5' NTR with that of HCV (Fig. 2A) while the coding sequence and 3' NTR of p5'HCV are identical to pBVDV. Positioning of the HCV 5' NTR in such a manner was necessary since translation initiation from the HCV IRES begins at or near the AUG start codon (Honda et al., 1996a; Reynolds et al., 1995; Reynolds et al., 1996; Rijnbrand et al., 1996). The specific infectivity of 5'HCV RNA synthesized in vitro was compared to that of BVDV RNA by transfection of MDBK (bovine kidney) cells (Fig. 2A). The specific infectivity of BVDV RNA was approximately  $4 \times 10^6$  plaque forming units (PFU)/ $\mu$ g RNA. In contrast, the specific infectivity of 5' HCV RNA was near the limit of detection (30-50 PFU/ $\mu$ g RNA) and considerable plaque heterogeneity was apparent. These results suggested that the HCV 5' NTR replacement chimera might be incapable of efficient replication and plaque formation and that the plaque forming virus observed had arisen by secondary mutation(s). Sequence analysis of plaque-purified 5' HCV viruses presented below confirmed that the replicating pool of virus contained such pseudorevertants.

Next, the *in vitro* translation efficiency of these two RNAs in rabbit reticulocyte extracts was analyzed to test whether the defect in specific infectivity of 5' HCV RNA could be attributed to lower translation efficiency. Although the specific infectivity of 5' HCV RNA was reduced ~5 logs compared to BVDV RNA, its translation efficiency was only slightly 5 reduced, ~twofold (Fig. 3, lane 1 vs. lane 2). The apparent size of the N-terminal cleavage product, N<sup>PRO</sup>, was identical for both RNAs, suggesting that translation initiated with the correct AUG. These data are consistent with the hypothesis that the BVDV 5' NTR contains signals that are required for a step in replication other than translation which are not present in the 5' HCV chimera.

Given the low specific infectivity of 5' HCV RNA, an experiment was performed to 10 test the effect of placing the BVDV 5' NTR sequence upstream of the HCV 5' NTR, resulting in tandem BVDV and HCV 5' NTRs (called BVDV + HCV). This arrangement actually decreased translation efficiency (Fig. 3, lane 14 vs. lane 1) yet restored infectivity (Fig. 2A). The plaques produced by BVDV + HCV were also heterogeneous in size, indicating that this 15 virus was unstable. Upon passage, RT-PCR analysis indicated that pseudorevertants had indeed arisen in which portions of the BVDV and/or HCV 5' NTRs had been deleted (data not shown). These data show that sequences in the BVDV 5' NTR required for virus replication can function when placed upstream of a functional HCV IRES driving translation of the BVDV polyprotein.

20

#### **Hairpins B1' and B1 in conjunction with the HCV IRES are sufficient for stable and efficient BVDV replication**

The sequences within the BVDV 5' NTR that restored replication in the context of the HCV 5' NTR were mapped using three deletion variants. The deletion BVDV + HCVdelB3 25 removed a large portion of hairpin B3; the deletion within BVDV + HCVdelB2B3 removed hairpins B2 and B3, and the deletion within BVDV + HCVdelB1B2B3 removed hairpins B1, B2 and B3. The specific infectivities of RNAs from these deletion mutants were near that of BVDV RNA (Fig. 2). Upon passage of these viruses, RT-PCR analyses and sequencing indicated that BVDV + HCV delB3 and BVDV + HCVdelB2B3 were stably propagated and 30 produced homogeneous plaques slightly smaller than those of wild-type BVDV (data not shown). In contrast, BVDV + HCVdelB1B2B3 produced smaller heterogeneous plaques. Reverse transcription-polymerase chain reaction (RT-PCR) analysis and sequencing indicated that BVDV + HCVdelB1B2B3 underwent a reversion event described in more detail below. The translation efficiencies of these three RNAs (Fig. 3, lanes 9, 10, and 12) were similar to 35 BVDV + HCV RNA (Fig. 3, lane 14), indicating that the deleted portions (hairpins B1, B2,

and B3) are not required for translation in the BVDV + HCV chimera. These results show that B1' and B1 are the minimal elements sufficient for stable replication in conjunction with the HCV 5' NTR.

Having shown that B1' and B1 are sufficient for replication in conjunction with the HCV 5' NTR, we next conducted a deletion analysis to determine the sequences within the HCV 5' NTR of BVDV + HCV delB2B3 required for replication. A large portion of H1 was deleted in BVDV + HCV delB2B3H1, while both H1 and H2 were deleted in BVDV + HCV delB2B3H1H2. Of these two RNAs, only BVDV + HCV delB2B3H1 was as infectious as parental BVDV RNA (Fig. 2B). However, the BVDV + HCV delB2B3H1 virus produced smaller plaques than BVDV + HCV delB2B3, indicating that hairpin H1 may augment replication of the chimera. In contrast, BVDV + HCV delB2B3H1H2 RNA was not infectious (Fig. 2B) and was translated poorly (Fig. 3, lane 11). Diminished HCV IRES activity might be due to deletion of hairpin H2 or juxtaposition of BVDV hairpins B1' and B1 with H3. A third derivative of BVDV + HCV delB2B3, with a *Sma* I-*Sma* I deletion abrogating HCV IRES function by removing H3, was also not infectious (data not shown). Thus, a 5' NTR consisting of B1' and B1 and a functional HCV IRES is sufficient for stable BVDV replication in MDBK cells. Similar results were obtained in BT cells, another BVDV-permissive continuous bovine cell line (data not shown).

## 20 Replacement of the BVDV 5' NTR with the EMCV 5' NTR

The following experiment was performed to determine whether the BVDV 5' NTR could be replaced by the 5' NTR of a more phylogenetically distant virus, EMCV. A derivative of BVDV was created, called 5' EMCV, that contains an exact replacement of the BVDV 5' NTR with the EMCV 5' NTR plus an additional guanosine residue at the 5' terminus for more efficient transcription initiation of T7 polymerase (Fig. 4A). The specific infectivity of 5' EMCV RNA was more than three orders of magnitude lower than BVDV RNA, indicating that it was defective for replication, although its specific infectivity was higher than that of 5' HCV RNA (compare Figs. 4A and 2A). Similar to 5' HCV, 5' EMCV produced heterogeneous plaques, and sequence analysis indicated that pseudorevertants had arisen. The lower specific infectivity of 5' EMCV RNA was not likely because of a defect in translation, since the translation efficiency of 5' EMCV RNA was about threefold higher in vitro than that of BVDV RNA (Fig. 3, lane 20 vs. lane 19).

Similar to BVDV + HCV, it was also determined whether the BVDV 5' NTR at the 5' end of the 5' EMCV RNA would increase its specific infectivity. BVDV + EMCVdelA (Fig. 35 4A) contained the entire BVDV 5' NTR in tandem with the EMCV 5' NTR lacking a portion

of hairpin A. BVDV + EMCVdelA RNA had a specific infectivity near that of BDVD RNA (compare Figs. 4A and 2A) despite having a lower translation efficiency than 5' EMCV (Fig. 3, lane 21 vs. lane 20). Similar to the results with BVDV + HCV, this implicates the added BVDV 5' NTR sequence for a step in viral replication other than translation. Two derivatives 5 of BVDV + EMCVdelA that contain deletions of portions of the BDVD 5' NTR but maintain the sequence of B1' and B1, BDVD + EMCVdelB3A and BVDV + EMCVdelB2B3A (Fig. 4A), also were infectious. These derivatives had translation efficiencies near that of the parental BVDV + EMCVdelA (Fig. 3, compare lanes 15 and 16 with lane 21). This demonstrated that hairpins B1' and B1 were sufficient for replication in conjunction with a 10 large portion of the EMCV 5' NTR. Derivatives of BVDV + EMCVdelB3A or BVDV + EMCVdelB2B3A that contain further deletions of EMCV (BVDV - EMCVdelB3ABC and BVDV + EMCVdelB2B3ABC in particular) were translated efficiently (Fig. 3, lanes 17 and 18) and were infectious (Fig. 4B). This indicates that the chimeras did not require putative 15 EMCV RNA replication signals (Martin & Palmenberg, 1996). However, derivatives with deletions extending into the canonical EMCV IRES were not infectious. For example, BVDV + EMCVdelB3A-H and BVDV + EMCVdelB2B3A-H, in which a portion of hairpin H is deleted, were not infectious (Fig. 4B) and were inefficiently translated in vitro (Fig. 3, lanes 22 and 23). It should be noted that all of the BVDV + EMCV chimeras produced plaques of heterogeneous size, indicating some instability.

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#### **Relatively simple 5' NTR mutations are observed in adapted pseudorevertants**

As mentioned previously, BVDV + HCVdelB1B2B3 did not replicate stably as indicated by the heterogeneity in the size of plaques produced by this virus. Upon passage and selection of medium plaque-producing variants, 5' RACE analysis and sequencing 25 indicated that nt 1-26 had been deleted in the pseudorevertants, removing a large portion of B1' which was apparently deleterious in the absence of B1. This deletion results in the 5' terminal sequence 5'GUAUCG which is identical to the first six bases of BVDV genome RNA (Fig. 5) and is repeated at positions 27-32.

Analysis of the passaged 5' EMCV virus indicated that the replicating progeny had 30 also undergone a simple deletion of sequence at the 5' end to generate more efficiently replicating variants (Fig. 5). After electroporation, the 5' EMCV virus pool was passaged 5 times at a multiplicity of infection of 0.1-1 PFU/cell on MDBK or BT cells, and the 5' termini of three randomly picked plaques were sequenced. For all three plaques selected, nt 2-209 had been deleted, again creating a genome RNA with the 5' terminal tetranucleotide sequence 35 5'-GUAU.

Analysis of the 5' HCV progeny indicated that more complicated variants had arisen. Most small plaque-producing variants were unstable and quickly reverted to medium plaque-producing variants. However, one small plaque-producing variant and two stable medium plaque-producing variants were isolated. 5' terminal sequences of the variants were amplified by rapid amplification of cDNA ends (RACE) and cloned into a plasmid vector, and sequences for several independent colonies were determined. The sequence of three clones of the small plaque-producing virus (5'HCV.R1) contained a deletion of HCV sequence from nt 1-34 and an addition of the dinucleotides 5'-AU in two clones and 5'-GU in the third clone. This creates a 5' terminus of 5'-(G/A) UAA (Fig. 5B), reminiscent of the first three bases of the BVDV genome RNA (5'-GU). Both medium plaque variants appeared to have arisen by RNA recombination with non-viral sequences (Fig. 5). One medium plaque variant (5' HCV.R2) had deleted the first 21 bases of the HCV sequence and contained instead a heterologous sequence of 22 bases. BLAST searches revealed a perfect match between this sequence and a sequence in a human retina cDNA of unknown function (Tsp509I). The second medium plaque variant (5' HCV.R3) had also undergone a possible recombination event leading to the addition of 12 nt to the 5' end of the HCV sequence. Given its short length, multiple matches were found in the database with this sequence. As for the small plaque variant, sequencing of multiple clones revealed heterogeneity at the extreme 5' end, with either G or A identified as the 5' base. Remarkably, for both medium plaque variants, the fused heterologous sequence began with the tetranucleotide sequence 5'-(G/A) UAU (Fig. 5B). For all three variants, sequencing of the entire 5' NTR and a portion of the N<sup>pro</sup> coding region revealed only these changes at the 5' termini.

#### 5' NTR sequence changes are sufficient for the pseudorevertant phenotypes

To assess the importance of these alterations at the 5' terminus of the 5' HCV pseudorevertants, derivatives of 5' HCV were created with the changes determined by 5' RACE (Fig. 6A) and analyzed the specific infectivities of these RNAs (Fig. 6B). Corresponding to the small plaque variant, a derivative called 5' HCV.R1 orig was engineered which contained a 5' NTR consisting of the dinucleotide 5'-GU at the 5' terminus of HCV nt 35-341. This results in a 5' terminus consisting of 5'-GUAA. 5'HCV.R1 orig RNA had a specific infectivity at least four orders of magnitude higher than 5' HCV RNA (Figs. 6B and 2A). This demonstrates that this 5' NTR structure is sufficient for phenotypic reversion to high specific infectivity. However, small plaques and considerable heterogeneity were observed for 5'HCV.R1 orig suggesting that additional mutations may be present in the original small plaque variant.

The engineered derivative 5'HCV.R2orig had a 5' NTR consisting of 22 nt of Tsp509I-homologous sequence followed by HCV nt 22-341. Another construct, called 5'HCV.R3orig was made, which has the 12 nt of the other heterologous sequence fused to the intact HCV 5' NTR. Specific infectivities for both these derivatives were essentially the same 5 as observed for wild type BVDV RNA ( $2\text{-}4 \times 10^6$  PFU/ $\mu\text{g}$ ; Fig. 6B). Transfection with these transcripts produced medium plaques, as observed for the original variants, and this phenotype was stable upon passaging. These results show that the altered 5'NTR sequences were responsible for the pseudorevertant phenotypes rather than changes elsewhere in their genomes.

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**Addition of the tetranucleotide sequence 5'-GUAU to the HCV 5' NTR allows efficient 15 BVDV replication**

For all three 5' HCV variants studied, as well as the BVDV + HCV delB1B2B3 and 5'EMCV pseudorevertants, 5' NTR alterations seemed to involve creation of a three- or four-base "consensus" sequence identical to the 5' terminus of BVDV genome RNA. To test the importance of this sequence, as opposed to fused heterologous sequences, we created a set of 20 variants with the BVDV 5' tetranucleotide sequence linked to the HCV 5' NTR or the deletion/recombinant break points identified during sequence analysis of the 5' HCV pseudorevertants (Fig. 6A). 5'HCV.R1cons had the tetranucleotide sequence 5'-GUAU fused to HCV nt 35-341. 5'HCV.R2cons had the 5'-GUAU tetranucleotide sequence fused to HCV nt 22-341. 5'HCV.R3cons contained the tetranucleotide sequence 5'-Guau fused to the intact 25 5' terminus of the HCV NTR. RNAs from all three of these derivatives had specific infectivities more than five orders of magnitude higher than 5'HCV and comparable to parental BVDV (Fig. 6B).

There were, however, significant differences between the phenotypes of some of these derivatives versus the reconstructed pseudorevertants. As mentioned above, 30 5'HCV.R1orig yielded tiny and small plaques and produced low virus yields even after 48 h. In contrast, the addition of four bases rather than two bases (5'-GUAU vs. 5'-GU) yielded virus with near wild-type plaque morphology (Fig. 6B) and growth Rates (Fig. 7). In the case of the smaller deletion, 5'HCV.R2orig and 5'HCV.R2cons were indistinguishable, suggesting that, other than the 5' four bases, the fused heterologous sequences were dispensable. This 35 was not the case, however, for the chimera containing the 5'-GUAU tetranucleotide sequence

fused to the intact HCV 5' NTR. 5'HCV.R3cons produced small plaques (Fig. 6B) and grew more slowly than 5'HCV.R3orig (Fig. 7) suggesting that the sequence/structure of the sequences downstream of the 5' four bases can affect replication efficiency.

5   **The tetranucleotide sequence 5'-GUAU is important for efficient BVDV RNA accumulation**

Next, the effects of the different 5' termini on virus-specific RNA accumulation directly after transfection were analyzed. This allowed a direct comparison between 5'HCV and the reconstructed pseudorevertants as well as selected BVDV + HCV deletion constructs.

10   MDBK cells were transfected with *in vitro* synthesized RNAs and labeled for 10 h beginning at 5 h post-transfection with  $^3$ H-UTP in the presence of actinomycin D (Fig. 8). RNA replication of the 5' HCV chimera was severely impaired to a level below detection (Fig. 8, lane 2). In contrast, every 5' NTR alteration of 5' HCV that increased RNA specific infectivity and allowed efficient virus growth led to readily detectable viral RNA

15   accumulation. Addition of B1' and B1 to the 5' terminus of the HCV 5' NTR restored RNA replication to a level ~50% of that observed for BVDV (BVDV + HCVdelB2B3; Fig. 8, lane 3 vs. lane 1). BVDV + HCVdelB2B3H1 displayed reduced RNA synthesis compared to BVDV + HCVdelB2B3 (Fig. 8, lane 4 vs. lane 3) perhaps explaining its small plaque phenotype and suggesting a possible positive role for H1 in replication of this chimera.

20   5'HCV.R1orig, which had exhibited plaque heterogeneity and slow growth, accumulated less RNA when compared to 5'HCV.R1cons (Fig. 8, lane 5 vs. lane 6). 5'HCV.R2orig and, 5'HCV.R2cons showed similar RNA accumulation (Fig. 8, lane 9 vs. lane 10) consistent with their medium plaque phenotypes; and 5'HCV.R3cons exhibited reduced RNA synthesis compared to 5'HCV.R3orig (Fig. 8, lane 8 vs. lane 7), consistent with their small-versus

25   medium-plaque phenotypes.

Although these RNA phenotypes are complex, the most striking result is that addition of the B1' B1 hairpins, addition of heterologous 5' sequences terminating with 5'-GUAU or simply fusion of this tetranucleotide sequence with the HCV 5' NTR or short 5' truncations of the HCV 5' NTR all dramatically upregulated RNA accumulation. This occurred without increasing translation efficiency, at least as measured in a cell-free assay (Fig. 3, compare lanes 3-8 to lane 1), suggesting that these sequences function at the level of RNA replication or stability.

### Discussion

The work presented here helps to define the requirements for a functional BVDV 5'NTR. The BVDV-specific 5' NTR sequences required for efficient replication in cell culture are minimal and consist of the 5' terminal sequence, 5'-GUAU. The sequence 5'-AUAU, detected for some pseudorevertants, may also be functional but this was not tested for technical reasons. This simple 5'-terminal tetranucleotide sequence, which is conserved among pestiviruses (Ruggli et al., 1996; Becher et al., 1998), was shown to function in the context of functional IRES elements derived from the hepacivirus HCV or the picornavirus EMCV. As discussed below, this may indicate that the 5' signals required for BVDV RNA replication are rather simple or that elements in these heterologous IRESs can functionally replace deleted BVDV sequences.

Sequences at the extreme 5' end of BVDV genome RNA could modulate the efficiency of RNA accumulation by affecting RNA stability, translation, promoter efficiency, or some combination of these processes. At this time, we can not distinguish among these possibilities but favor an effect on RNA replication. The complement of the BVDV 5' sequence at the 3' end of the negative-strand RNA presumably functions in the initiation of positive-strand RNA synthesis. Thus, AUAC-3' at the 3' terminus of minus-strand RNA may be important for positive-strand RNA synthesis. Interestingly, for some positive-strand RNA viruses such as rubella virus (Pugachev & Frey, 1998), flock house virus (Ball, 1994) and turnip crinkle virus (Guan et al., 1997), only minimal *cis*-acting sequences at the 3' termini of negative-strand RNAs are required positive-strand RNA synthesis. In contrast to the 5' NTR replacements, we were unable to generate replication-competent BVDV-HCV replacing that of BVDV (data not shown). This may indicate that the signals within the pestivirus 3' NTR required for initiation of negative-strand RNA synthesis are more complex and virus specific. Once the replication complex has assembled at the 3' NTR and transversed the RNA during negative-strand synthesis, the requirements of the 5' NTR for initiation of positive-strand synthesis may be minimal.

Although the RNA replication signals within the 5' NTR appear to be rather simple, it is possible that the signals important for RNA replication actually extend into the IRES and are more complicated. For instance, the 5'HCV pseudorevertants were more stable and grew to higher titers than the 5'EMCV counterparts, despite the fact that the 5'EMCV RNAs were translated more efficiently *in vitro*. This may indicate that the BVDV and HCV IRESs contain signals important for RNA synthesis that are absent in the EMCV IRES.

It is perhaps not surprising that 5' HCV appeared to recombine with cellular mRNAs to acquire a 5' terminus with the 5' -(G/A) UAU consensus, given that non-cytopathic strains

of BVDV can recombine with BVDV RNA or cellular mRNAs to generate cytopathic strains of BVDV (Meyers & Thiel, 1996). Presumably, this recombination event involves template switching during negative-strand RNA synthesis, as observed for polio-virus (Kirkegaard & Baltimore, 1986). In contrast to 5' HCV, simple deletions of 5' terminal viral sequences could 5 account for the BVDV + HCVdelB1B2B3 and 5'EMCV pseudorevertants since the tetranucleotide sequence is present in these 5' NTRs upstream of functional IRES elements. Such deletions could occur by partial degradation of positive-strand template prior to negative-strand synthesis, by premature termination during negative-strand RNA synthesis, or by degradation of 3' terminal negative-strand sequence after synthesis. It is proposed that 10 5'HCV was forced to recombine with cellular sequences because HCV does not have an 5'- (G/A) UAU sequence upstream of its IRES. The first occurrence of an (G/A)UAUA tetranucleotide sequence is at nt 94-97 within hairpin H2, and a 5' deletion extending into this sequence would presumably inactivate or severely impair HCV IRES activity. It is interesting that BVDV + HCVdelB1B2B3 and 5'EMCV pseudorevertants were generated at much higher 15 frequency than 5'HCV pseudorevertants. This may indicate that recombination between BVDV and cellular RNAs is a rare event compared to the processes which lead to deletion of terminal viral sequences.

Poliovirus chimeras dependent upon a functional HCV IRES have been reported (Lu & Wimmer, 1996). Interestingly, viable poliovirus chimeras were produced only when HCV 20 sequences included both the IRES and the N-terminal portion of the HCV ORF. Nucleotide sequences or structures in the downstream ORF can modulate HCV IRES translational efficiency (see Reynolds et al., 1995; Honda et al., 1996a) but it was also suggested that the N-terminal portion of the HCV core polypeptide might be involved. In the case of our 5' HCV pseudorevertants, there is no requirement for HCV C protein sequences. Although the 25 translation efficiency of the HCV IRES in the presence of additional HCV sequences 3' to the AUG start was not directly assessed, the HCV chimeras and pseudorevertants were translationally active and infectious in the absence of any portion of the HCV ORF. This indicates that either the HCV IRES does not extend into the HCV ORF or that the BVDV ORF contains analogous sequence which functions in our 5'HCV chimeras. There is some 30 limited identity between HCV and BVDV within this region. For example, HCV nt 359-394 and BVDV nt 405-440 are identical at 21 of 36 positions, although identity within this sequence may be attributed to a high adenosine content. It is interesting to note that the luciferase (LUC) and chloramphenicol acetyl transferase (CAT) reporter genes previously used to detect HCV IRES activity (Tsukiyama-Kohara et al., 1992; Wang et al., 1993) also 35 have adenosine- or purine-rich regions in relatively the same position as the HCV ORF and

BVDV ORF. If this region is indeed important for IRES activity, this may explain why some have observed that the HCV IRES does not require a portion of the HCV ORF for translation of CAT or LUC (Tsukiyama-Kohara et al., 1992; Wang et al., 1993). Point mutations and insertions within this region of HCV have been shown to reduce HCV IRES activity in vitro  
5 (Honda et al., 1996a,b).

Despite the fact that B1' and B1 are conserved among different strains of BVDV and similar hairpins are present in border disease virus and CSFV (Deng & Brock, 1993; Becher et al., 1998), B1' and B1 were dispensable for BVDV replication, provided that the 5' tetranucleotide sequence 5'-(G/A)UAU remained. This may indicate a role for B1' and B1 in  
10 viral replication in vivo that we do not observe in cell culture. It will be interesting to test the phenotype of chimeras that lack B1' and B1 in vivo to determine if they are attenuated and might serve as useful BVDV vaccines. In this vein, several studies with flaviviruses have demonstrated that alterations in 5' NTR or 3' NTR elements can lead to attenuation in vivo  
15 (Cahour et al., 1995; Men et al., 1996; Mandl et al., 1998). BVDV chimeras that utilize the HCV or EMCV IRES may also prove to be attenuated simply due to the presence of the heterologous IRES. For poliovirus, it has been shown that differences in IRES efficiency in  
20 different host-cell environments can modulate host range and virulence (Shiroki et al., 1997).

BVDV-HCV chimeras that are dependent on a functional HCV IRES may have another practical application. It may be possible to use these chimeras to screen for anti-HCV  
25 therapeutics that target the HCV IRES. Other researchers have shown antisense oligonucleotide-mediated inhibition of HCV gene expression in hepatocytes by targeting the oligonucleotides to the HCV IRES (Hanecak et al., 1996). It will be of interest to measure the efficacy of antisense oligonucleotides or ribozymes (Lieber et al., 1996) against replicating virus, and these chimeras are more useful than HCV for this purpose since they are able to replicate efficiently in cell culture. BVDV is believed to be a reasonable model of HCV  
25 replication not only because of homology and conserved motifs within the 5' NTR but also because of similarities in overall genetic organization (Rice, 1996) and polyprotein processing strategy (Tautz et al., 1997; Xu et al., 1997).

In view of the above, it will be seen that the several advantages of the invention are  
30 achieved and other advantageous results attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

All references cited in this specification, including patents and patent applications, are hereby incorporated by reference. The discussion of references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of  
5 the cited references.

**What is Claimed is:**

1. A polynucleotide comprising a chimeric viral RNA which comprises:
  - (a) a 5' nontranslated region (5' NTR);
  - (b) an open reading frame (ORF) region; and
  - (c) a 3' nontranslated region (3' NTR);5 wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV), and wherein said chimeric viral RNA is replication-competent.
- 10 2. The polynucleotide of claim 1, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).
- 15 3. The polynucleotide of claim 2, wherein the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAU.
4. The polynucleotide of claim 3, wherein the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).
- 20 5. The polynucleotide of claim 4, wherein the ORF and the 3' NTR consist of second and third BVDV sequences.
6. The polynucleotide of claim 5, wherein the 5' terminal sequence comprises 5' GUAU.
- 25 7. The polynucleotide of claim 4, wherein the ORF comprises a second HCV sequence encoding at least one structural protein operably linked to a second BVDV sequence.
- 30 8. The polynucleotide of claim 1, wherein the pestivirus is BVDV and the chimeric region is the 3' NTR.
9. The polynucleotide of claim 8, wherein the first HCV sequence in the chimeric 3' NTR comprises the HCV 98 bp 3' terminal element (SEQ ID NO:X) operably linked to the first BVDV sequence.

10. A method for identifying compounds having antiviral activity against hepatitis C virus (HCV) comprising the steps of:

(a) providing a first cell containing a chimeric viral RNA which is replication-competent in the cell, the chimeric viral nucleic acid comprising a 5' nontranslated region (5' NTR), an open reading frame (ORF) region; and a 3' nontranslated region (3' NTR);  
5 wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV);

10 (b) providing a second cell containing the pestivirus; and  
(c) comparing the replication efficiency of the chimeric viral RNA acid in the presence and absence of a test compound to the replication efficiency of the pestivirus in the presence and absence of the test compound,  
wherein a greater reduction in compound-induced replication efficiency of the chimeric viral RNA than the pestivirus indicates the compound has anti-HCV activity.

15

11. The method of claim 10, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).

20

12. The method of claim 11, wherein the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAU.

13. The method of claim 12, wherein the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

25

14. The method of claim 13, wherein the ORF and the 3' NTR comprise second and third sequences from the BVDV.

15. The method of claim 10, wherein the pestivirus is BVDV and the chimeric region is the 3' NTR.

30

16. A genetically-engineered virus comprising a chimeric RNA genome which comprises:  
(a) a 5' nontranslated region (5' NTR);  
(b) an open reading frame (ORF) region; and  
35 (c) a 3' nontranslated region (3' NTR);

wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV); and wherein said chimeric RNA genome is replication-competent.

5        17.      The genetically-engineered virus of claim 16, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).

10       18.      The genetically-engineered virus of claim 16, wherein the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAU and the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

15       19.      A vaccine against bovine viral diarrhea virus (BVDV) comprising an immunogenically-effective amount of a genetically-engineered virus comprising a chimeric RNA genome having:

- (a) a 5' nontranslated region (5' NTR);
- (b) an open reading frame (ORF) region; and
- (c) a 3' nontranslated region (3' NTR);

20       wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from BVDV in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV), and wherein the genetically-engineered virus is attenuated as compared to BVDV.

25       20.      The vaccine of claim 19, wherein the chimeric region is the 5' NTR and the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAU and the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

30       21.      A polynucleotide comprising a chimeric viral RNA which comprises:  
(a) a 5' nontranslated region (5' NTR);  
(b) an open reading frame (ORF) region; and  
(c) a 3' nontranslated region (3' NTR);

35       wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a heterologous nucleotide sequence and wherein said chimeric viral RNA is replication-competent.

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EMCV

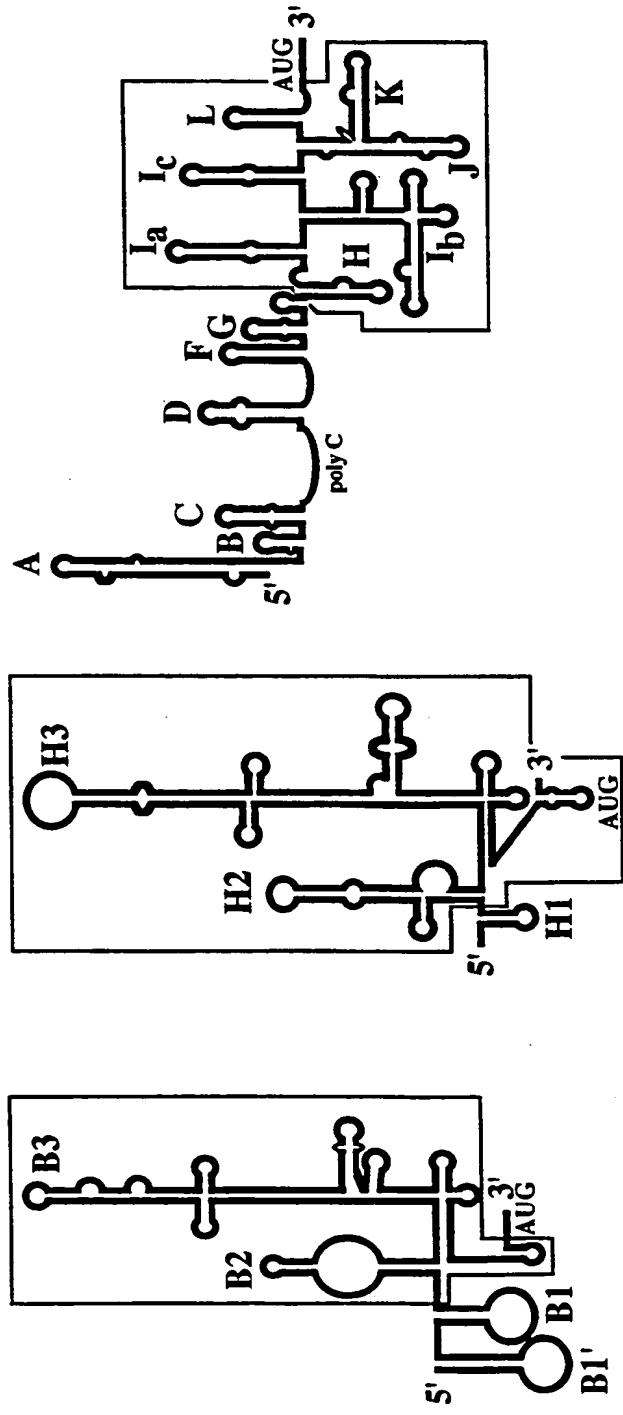
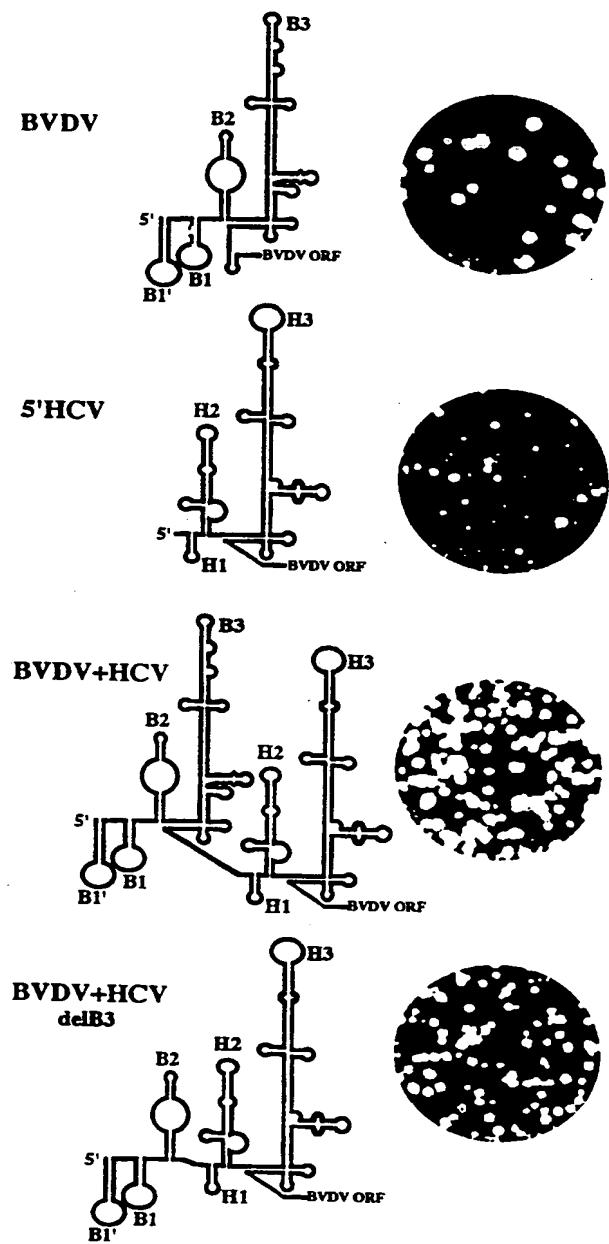


FIGURE 1

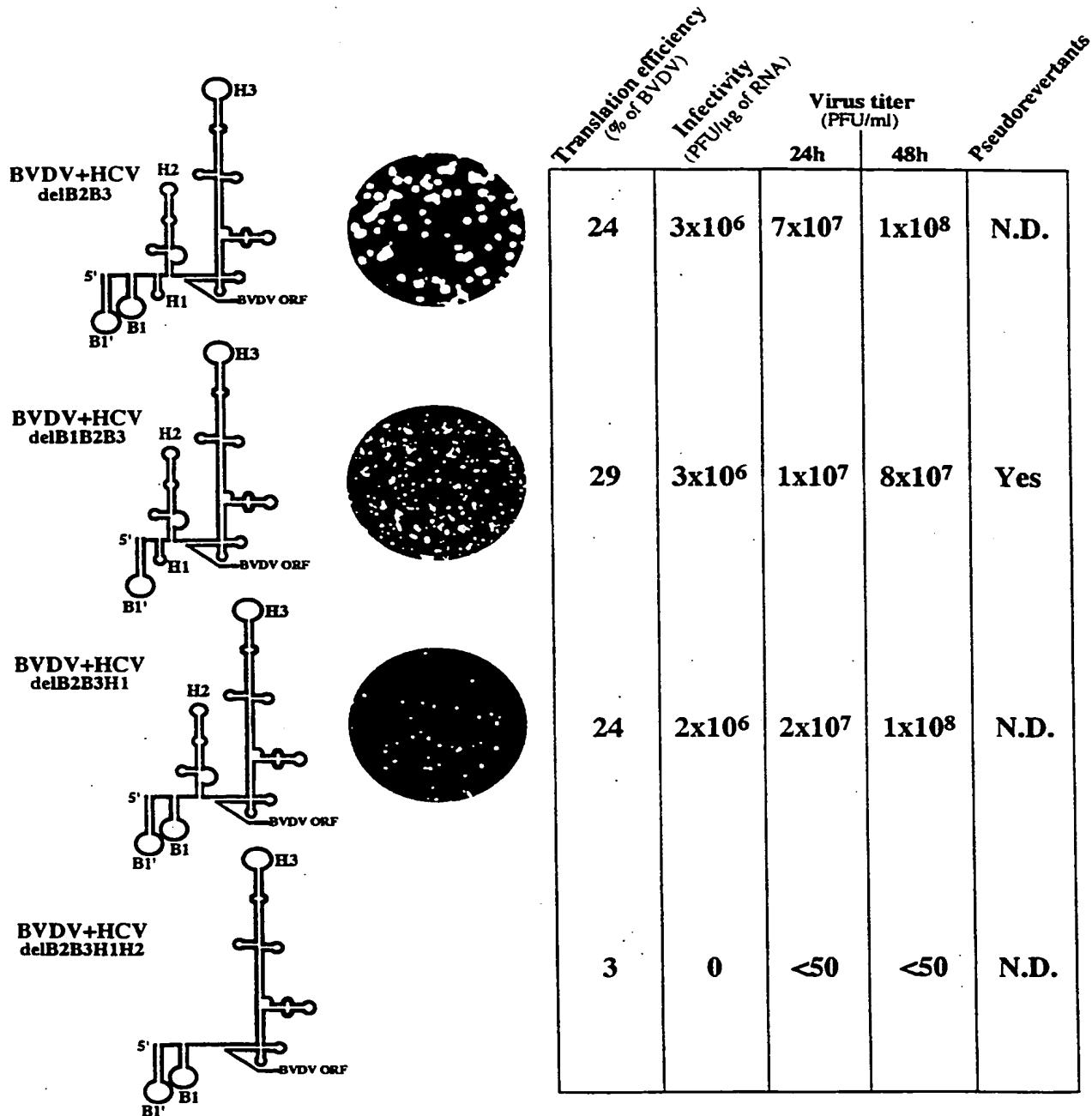
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|                       | Translation efficiency<br>(% of BVDV) | Infectivity<br>(PFU/ $\mu$ g of RNA) | Virus titer<br>(PFU/ml) |                           | Pseudorevertants |
|-----------------------|---------------------------------------|--------------------------------------|-------------------------|---------------------------|------------------|
|                       |                                       |                                      | 24 h                    | 48 h                      |                  |
| <b>BVDV</b>           | 100                                   | $4 \times 10^6$                      | $1 \times 10^8$         | $8 \times 10^7$           | No               |
| <b>5'HCV</b>          | 50                                    | 30-50                                | <50                     | $4 \times 10^5$<br>(72 h) | Yes              |
| <b>BVDV+HCV</b>       | 25                                    | $5 \times 10^6$                      | $1 \times 10^8$         | $1 \times 10^8$           | Yes              |
| <b>BVDV+HCV delB3</b> | 16                                    | $4 \times 10^6$                      | $7 \times 10^7$         | $1 \times 10^8$           | N.D.             |

FIGURE 2A

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**FIGURE 2B**

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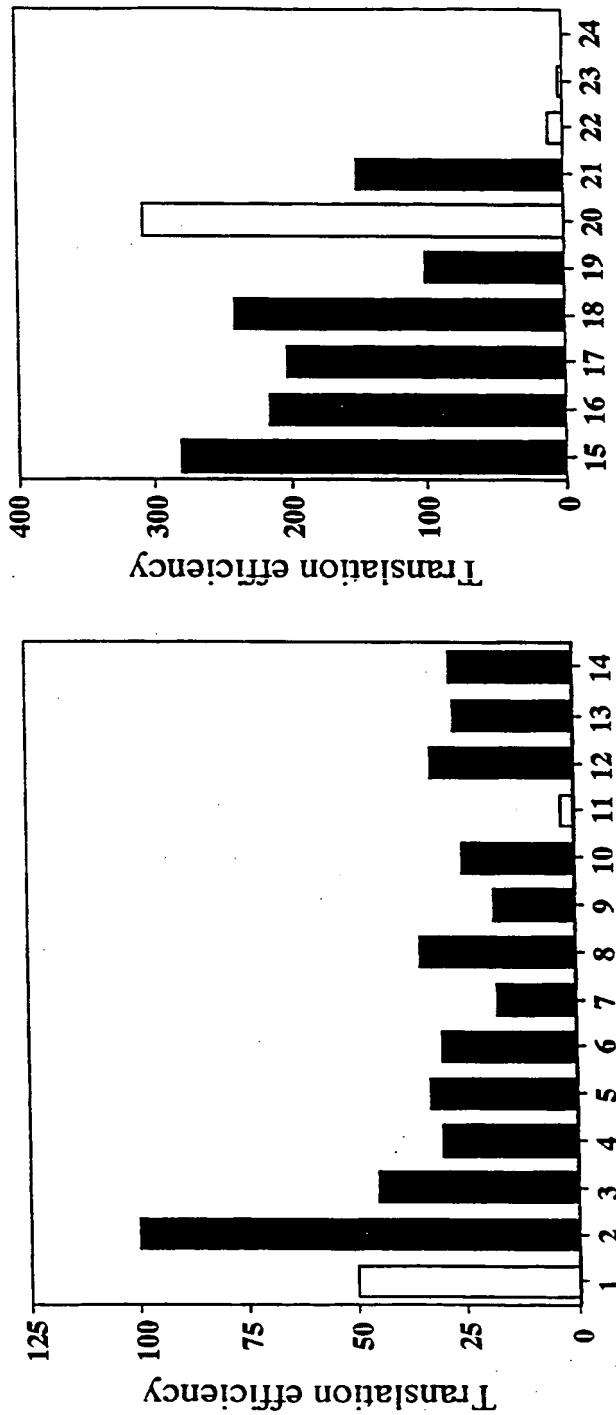


FIGURE 3

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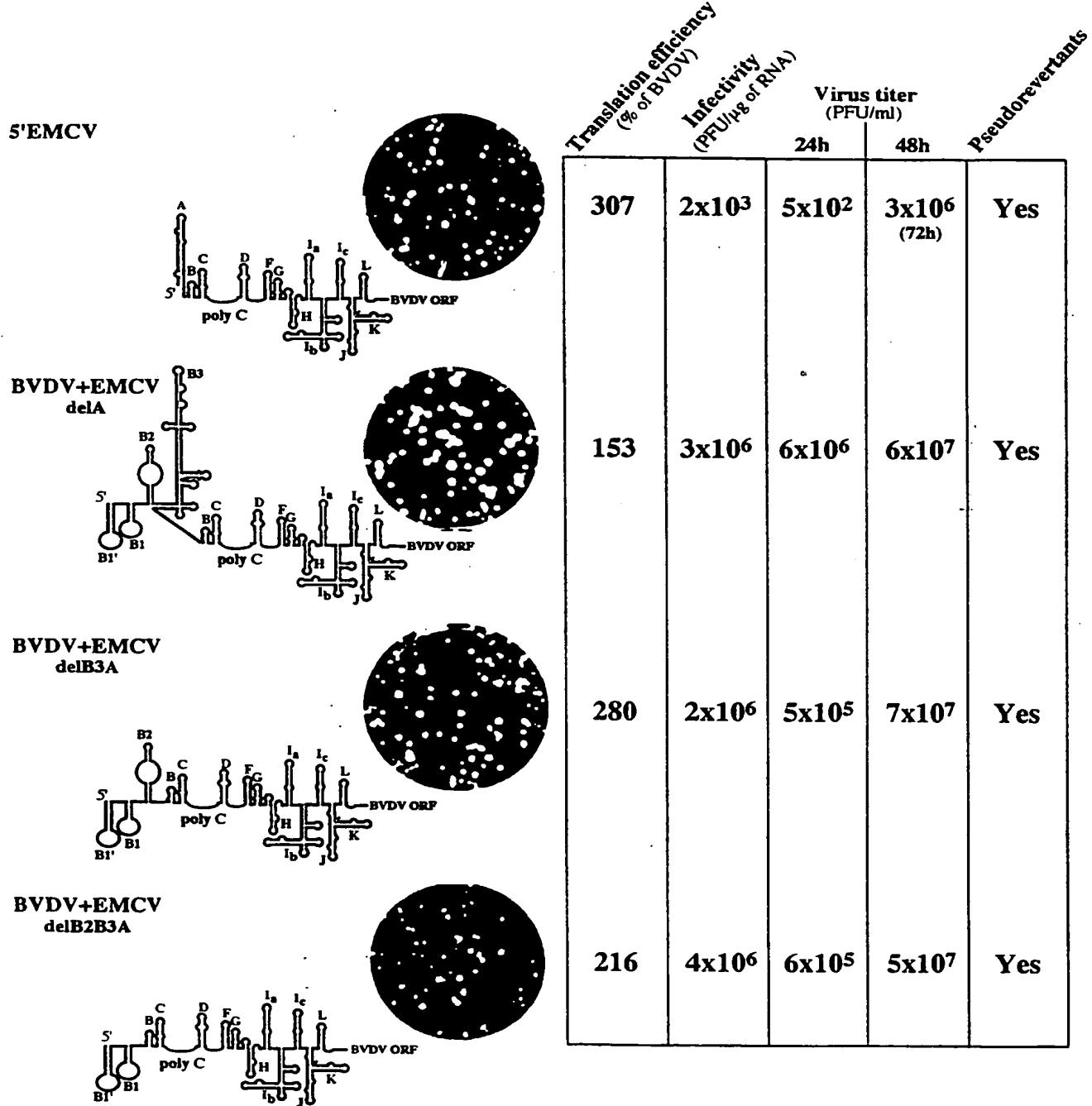


FIGURE 4A

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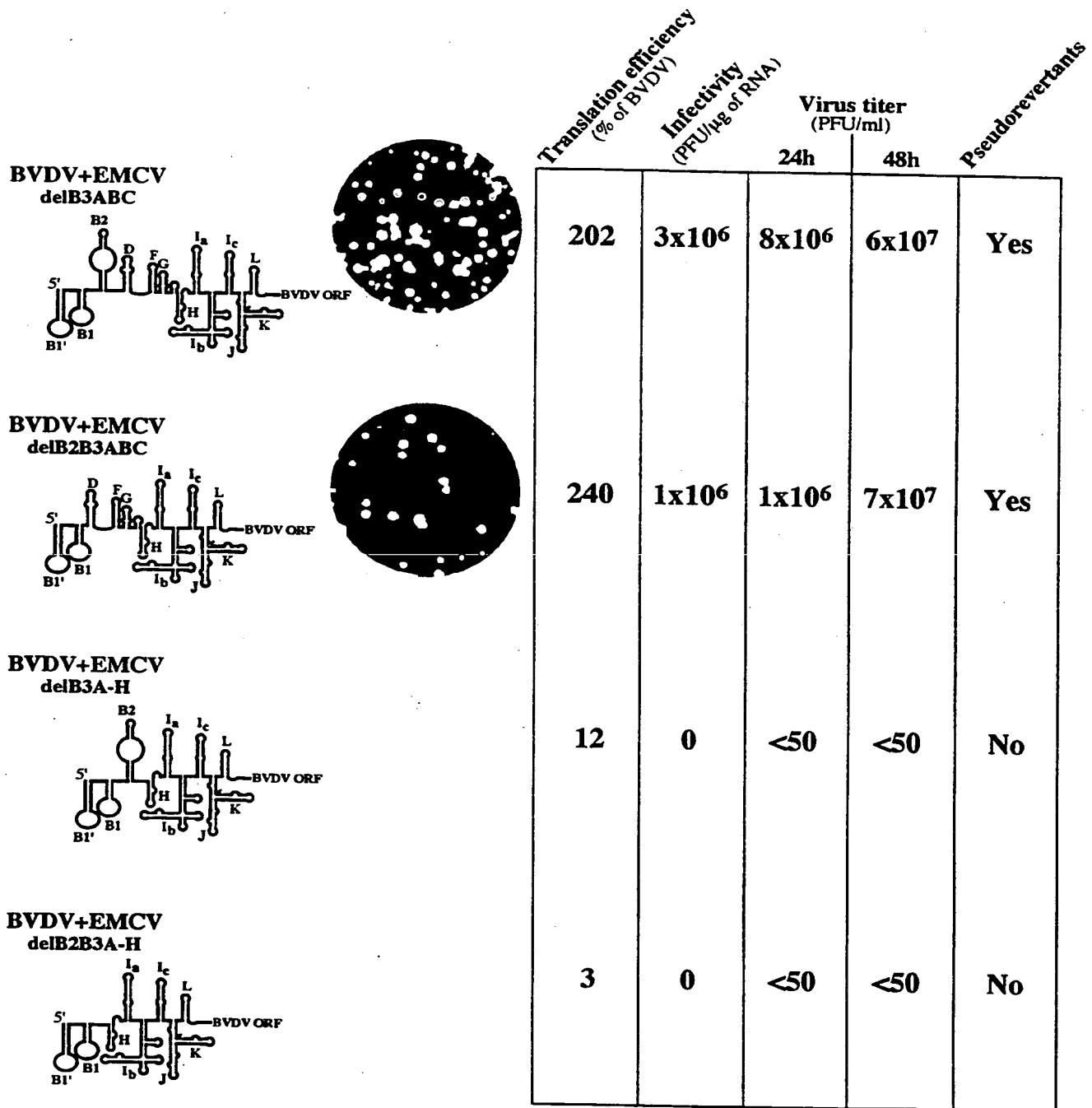


FIGURE 4B

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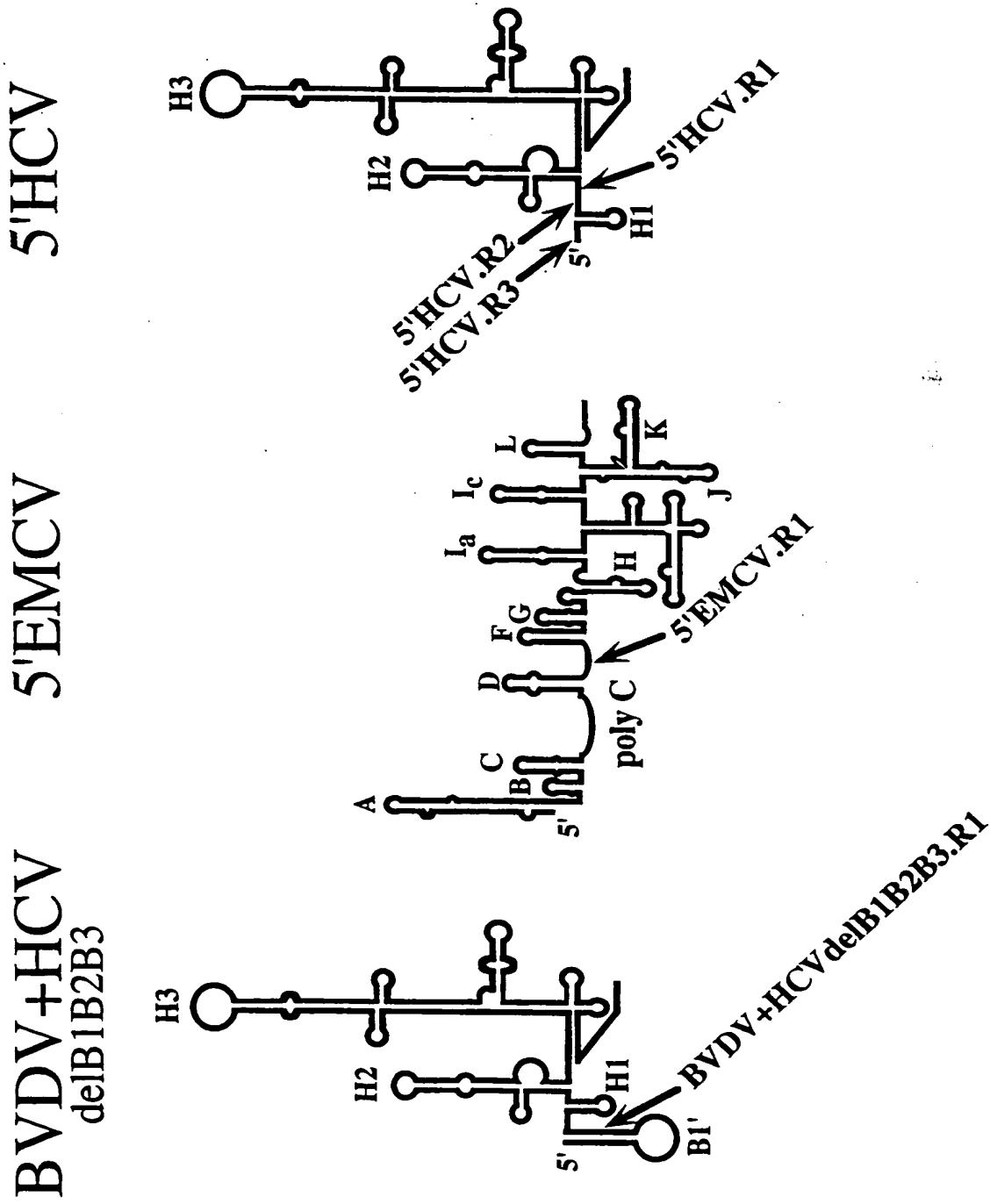


FIGURE 5A

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2

**FIGURE 5B**

A

5' HCV  
5' HCV.R1orig  
5' HCV.R2cons  
5' HCV.R3orig  
5' HCV.R3cons

gccaggcccugauuggggcgacacuccaccuaucacuccugugaggaacu  
GUauacacuccccugugaggaacu  
**GUAU**auacacuccaaugaaucacuccaccuaucacuccugugaggaacu  
GUAUCAGGAAGUGCCAAUGCUGAAcacuccaccuaucacuccugugaggaacu  
GUAUACUCCACCAGGGCGACACUCCACCUCCUGUGAGGAACU  
GUAUGGGTUGCCAGCCCCUGAUGGGGCGACACUCCACCUCCUGUGAGGAACU  
GUAUGCCAGCCCCUGAUGGGGCGACACUCCACCAUGAAUCACUCCUGUGAGGAACU

FIGURE 6A

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|  | Translation<br>efficiency<br>(% of BVDV) | Infectivity<br>(PFU/ $\mu$ g of RNA) | Virus titer (PFU/ml) |                 |
|--|--|--------------------------------------|----------------------|-----------------|
|  |  |                                      | 24h                  | 48h             |
| BVDV   | 100                                      | $4 \times 10^6$                      | $7 \times 10^7$      | $1 \times 10^8$ |
| 5'HCV.R1orig<br>(5' -GUAA)                   | 45                                       | $4 \times 10^5$                      | $2 \times 10^3$      | $2 \times 10^5$ |
| 5'HCV.R1cons<br>(5' -GUAU)                   | 29                                       | $3 \times 10^6$                      | $4 \times 10^7$      | $5 \times 10^7$ |
| 5'HCV.R2orig<br>(5' -GUAUCAGAAGUGCGAAUGCUGA) | 17                                       | $2 \times 10^6$                      | $7 \times 10^6$      | $5 \times 10^7$ |
| 5'HCV.R2cons<br>(5' -GUAU)                   | 35                                       | $3 \times 10^6$                      | $2 \times 10^7$      | $4 \times 10^7$ |
| 5'HCV.R3orig<br>(5' -GUAUUGCAGUUU)           | 33                                       | $3 \times 10^6$                      | $4 \times 10^7$      | $5 \times 10^7$ |
| 5'HCV.R3cons<br>(5' -GUAU)                   | 30                                       | $3 \times 10^6$                      | $1 \times 10^7$      | $6 \times 10^7$ |

FIGURE 6B

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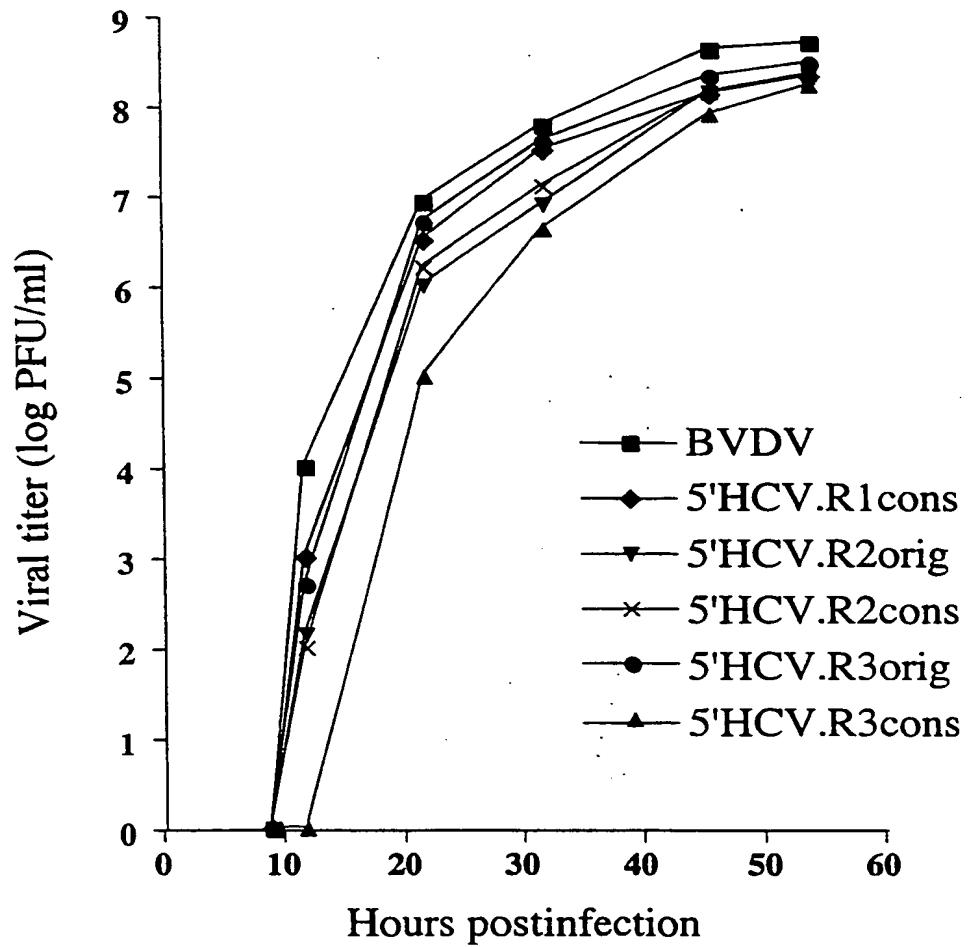
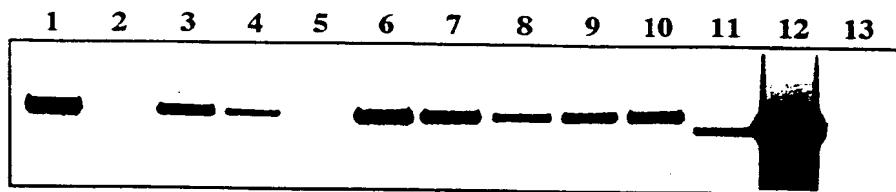
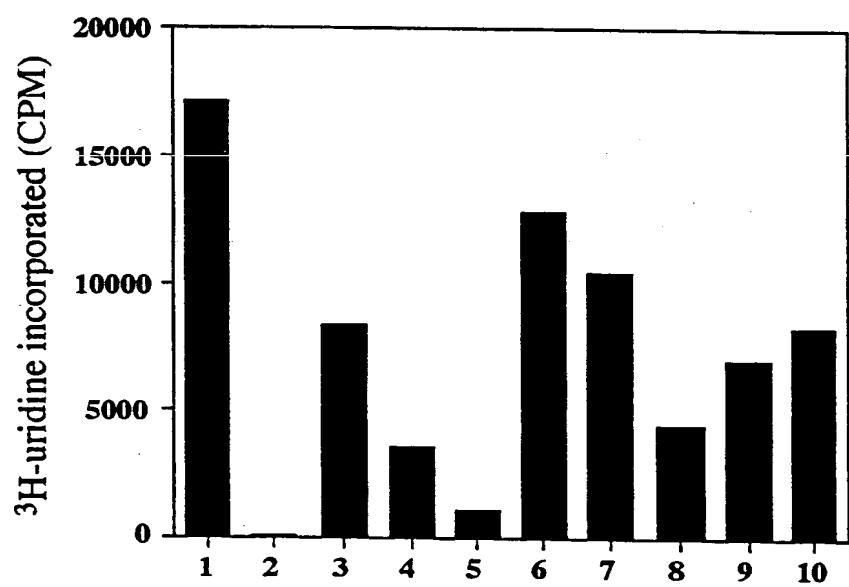


FIGURE 7

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**A****B****FIGURE 8**

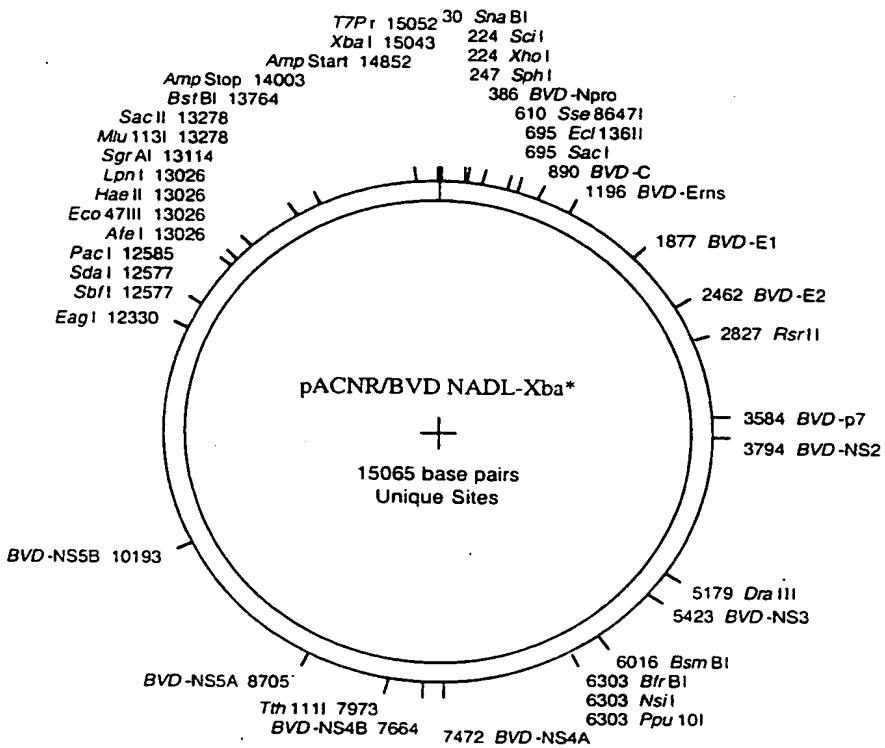
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**pACNR/BVD NADL-Xba\* -> Graphic Map**

DNA sequence 15065 bp gatacggagaat ... cgactcaactata circular

pACNR/BVD NADL-Xba = HaeII and XbaI digest of pACNR/BVD NADL ligated to  
 HaeII and XbaI digest of pACNR1180/DraIII-/BVD5  
 8/27 corrected nt 12136 G to C to give HpaI site.

Co

**FIGURE 9**

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## pACNR/BVD NADL-Xba\* -&gt; Genes

DNA sequence 15065 b.p. gtatacgagaat ... cgactcactata circular

pACNR/BVD NADL-Xba = HaeII and XbaI digest of pACNR/BVD NADL ligated to  
 HaeII and XbaI digest of pACNR1180/DraIII-/BVDS.  
 8/27 corrected nt 12136 G to C to give HpaI site.

Co

```

1 gtatacgagaattagaaaaggcactcgatatacgatggcaattaaaaataataattaggcctagggaaacaatccctc 80
81 tcagcgaaggccgaaaagaggctagccatgcccttaggactagcataatgagggggtagcaacagtggtagttcg 160
161 ttggatggcttaagccctgagtagcagggttagtcgtcgtgggtcgacgccttggaaataaaaggctcgagatgccacgtgg 240
241 acgaggccatgccccaaagcacatcttaacctgagcgggggtcgcccaggtaaaagcagtttaaccgactgttacaata 320
321 cagcctgatagggtgctgcagaggcccactgtattgtactaaaaatctctgttacatggcac ATG GAG TTG 394
1 M E L 3
395 ATC ACA AAT GAA CTT TTA TAC AAA ACA TAC AAA CAA AAA CCC GTC GGG GTG GAG GAA CCT 454
4 I T N E L L Y K T Y K Q K P V G V E E P 23
455 GTT TAT GAT CAG GCA GGT GAT CCC TTA TTT GGT GAA AGG GGA GCA GTC CAC CCT CAA TCG 514
24 V Y D Q A G D P L F G E R G A V H P Q S 43
515 ACG CTA AAG CTC CCA CAC AAG AGA GGG GAA CGC GAT GTT CCA ACC AAC TTG GCA TCC TTA 574
44 T L K L P H K R G E R D V P T N L A S L 63
575 CCA AAA AGA GGT GAC TGC AGG TCG GGT AAT AGC AGA GGA CCT GTG AGC GGG ATC TAC CTG 634
64 P K R G D C R S G N S R G P V S G I Y L 83
635 AAG CCA GGG CCA CTA TTT TAC CAG GAC TAT AAA GGT CCC GTC TAT CAC AGG GCC CCG CTG 694
84 K P G P L F Y Q D Y K G P V Y H R A P L 103
695 GAG CTC TTT GAG GAG GGA TCC ATG TGT GAA AGC ACT AAA CGG ATA GGG AGA GCA ACT GGA 754
104 E L F E E G S M C E T T K R I G R V T G 123
755 AGT GAC GGA AAG CTG TAC CAC ATT TAT GTG TGT ATA GAT GGA TGT ATA ATA ATA AAA AGT 814
124 S D G K L Y H I Y V C I D G C I I I K S 143
815 GCC ACG AGA AGT TAC CAA AGG GTG TTC AGG TGG GTC CAT AAT AGG CTT GAC TGC CCT CTA 874
144 A T R S Y Q R V F R W V H N R L D C P L 163
875 TGG GTC ACA ACT TGC TCA GAC ACG AAA GAA GAG GGA GCA ACA AAA AGG AAA ACA CAG AAA 934
164 W V T T C S D T K E E G A T K K K T Q K 183
935 CCC GAC AGA CTA GAA AGG GGG AAA ATG AAA ATA GTG CCC AAA GAA TCT GAA AAA GAC AGC 994
184 P D R L E R G K M K I V P K E S E K D S 203
995 AAA ACT AAA CCT CCG GAT GCT ACA ATA GTG GTG GAA GGA GTC AAA TAC CAG GTG AGG AAG 1054
204 K T K P P D A T I V V E G V K Y Q V R K 223
1055 AAG GGA AAA ACC AAG AGT AAA AAC ACT CAG GAC GGC TTG TAC CAT AAC AAA AAC AAA CCT 1114
224 K G K T K S K N T Q D G L Y H N K N K P 243
1115 CAG GAA TCA CGC AAG AAA CTG GAA AAA GCA TTG TTG GCG TGG GCA ATA ATA GCT ATA GTT 1174
244 Q E S R K K L E K A L L A W A I I A I V 263
1175 TTG TTT CAA GTT ACA ATG GGA GAA AAC ATA ACA CAG TGG AAC CTA CAA GAT AAT GGG ACG 1234
264 L F Q V T M G E N I T Q W N L Q D N G T 283
1235 GAA GGG ATA CAA CGG GCA ATG TTC CAA AGG GGT GTG AAT AGA AGT TTA CAT GGA ATC TGG 1294
284 E G I Q R A M F Q R G V N R S L H G I W 303
1295 CCA GAG AAA ATC TGT ACT GGT GTC CCT TCC CAT CTA GCC ACC GAT ATA GAA CTA AAA ACA 1354
304 P E K I C T G V P S H L A T D I E L K T 323
1355 ATT CAT GGT ATG ATG GAT GCA AGT GAG AAG ACC AAC TAC AGC TGT TGC AGA CTT CAA CGC 1414
324 I H G M M D A S E K T N Y T C C R L Q R 343
1415 CAT GAG TGG AAC AAG CAT GGT TGG TGC AAC TGG TAC AAT ATT GAA CCC TGG ATT CTA GTC 1474
344 H E W N K H G W C N W Y N I E P W I L V 363
1475 ATG AAT AGA ACC CAA GCC AAT CTC ACT GAG GGA CAA CCA CCA AGG GAG TGC GCA GTC ACT 1534
364 M N R T Q A N L T E G Q P P R E C A V T 383
1535 TGT AGG TAT GAT AGG GCT AGT GAC TTA AAC GTG GTA ACA CAA CCT AGA GAT AGC CCC ACA 1594
384 C R Y D R A S D L N V V T Q A R D S P T 403
1595 CCC TTA ACA GGT TGC AAG AAA GGA AAG AAC TTC TCC TTT GCA GGC ATA TTG ATG CGG GGC 1654
404 P L T G C K K G K N F S F A G I L M R G 423

```

FIGURE 10-1

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|   |
|---|
| 1655 CCC TGC AAC TTT GAA ATA GCT GCA AGT GAT GTA TTA TTC AAA GAA CAT GAA CGC ATT AGT 1714 |
| 424 P C N F E I A A S D V L F K E H E R I S 443   |
| 1715 ATG TTC CAG GAT ACT ACT CTT TAC CTT GTT GAC GGG TTG ACC AAC TCC TTA GAA GGT GCC 1774 |
| 444 M F Q D T T L Y L V D G L T N S L E G A 463   |
| 1775 AGA CAA GGA ACC CCT AAA CTG ACA ACC TGG TTA GGC AAG CAG CTC GGG ATA CTA GGA AAA 1834 |
| 464 R Q G T A K L T T W L G K Q L G I L G K 483   |
| 1835 AAG TTG GAA AAC AAG AGT AAG ACG TGG TTT GGA GCA TAC GCT GCT TCC CCT TAC TGT GAT 1894 |
| 484 K L E N K S K T W F G A Y A A S P Y C D 503   |
| 1895 GTC GAT CGC AAA ATT GGC TAC ATA TGG TAT ACA AAA AAT TGC ACC CCT GCC TGC TTA CCC 1954 |
| 504 V D R K I G Y I W Y T K N C T P A C L P 523   |
| 1955 AAG AAC ACA AAA ATT GTC CGC CCT GGG AAA TTT GAC ACC AAT GCA GAG GAC GGC AAG ATA 2014 |
| 524 K N T K I V G P G K F D T N A E D G K I 543   |
| 2015 TTA CAT GAG ATG GGG GGT CAC TTG TCG GAG GTA CTA CTA CTT TCT TTA GTG GTG CTG TCC 2074 |
| 544 L H E M G G H L S E V L L L S L V V L S 563   |
| 2075 GAC TTC GCA CGG GAA ACA GCT AGT GTA ATG TAC CTA ATC CTA CAT TTT TCC ATC CCA CAA 2134 |
| 564 D F A P E T A S V M Y L I L H F S I P Q 583   |
| 2135 AGT CAC GTT GAT GTA ATG GAT TGT GAT AAG ACC CAG TTG AAC CTC ACA GTG GAG CTG ACA 2194 |
| 584 S H V D V M D C D K T Q L N L T V E L T 603   |
| 2195 ACA GCT GAA GTA ATA CCA GGG TCG GTC TGG AAT CTA GGC AAA TAT GTA TGT ATA AGA CCA 2254 |
| 604 T A E V I P G S V W N L G K Y V C I R P 623   |
| 2255 AAT TGG TGG CCT TAT GAG ACA ACT GTA GTG TTG GCA TTT GAA GAG GTG AGC CAG GTG GTG 2314 |
| 624 N W W P Y E T T V V L A F E E V S Q V V 643   |
| 2315 AAG TTA GTG TTG AGG GCA CTC AGA GAT TTA ACA CGC ATT TGG AAC GCT GCA ACA ACT ACT 2374 |
| 644 K L V L R A L R D L T R I W N A A T T T 663   |
| 2375 GCT TTT TTA GTA TGC CTT GTT AAG ATA GTC AGG GGC CAG ATG GTA CAG GGC ATT CTG TGG 2434 |
| 664 A F L V C L V K I V R G Q M V Q G I L W 683   |
| 2435 CTA CTA TTG ATA ACA GGG GTA CAA GGG CAC TTG GAT TGC AAA CCT GAA TTC TCG TAT GCC 2494 |
| 684 L L L I T G V Q G H L D C K P E F S Y A 703   |
| 2495 ATA GCA AAG GAC GAA AGA ATT GGT CAA CTG GGG GCT GAA GCC CTT ACC ACC ACT TGG AAG 2554 |
| 704 I A K D E R I G Q L G A E G L T T T W K 723   |
| 2555 GAA TAC TCA CCT GGA ATG AAG CTG GAA GAC ACA ATG GTC ATT GCT TGG TGC GAA GAT GGG 2614 |
| 724 E Y S P G M K L E D T M V I A W C E D G 743   |
| 2615 AAG TTA ATG TAC CTC CAA AGA TGC ACG AGA GAA ACC AGG TAT CTC GCA ATC TTG CAT ACA 2674 |
| 744 K L M Y L Q R C T R E T R Y L A I L H T 763   |
| 2675 AGA GCC TTG CCG ACC AGT GTG GTA TTC AAA AAA CTC TTT GAT GGG CGA AAG CAA GAG GAT 2734 |
| 764 R A L P T S V V F K K L F D G R K Q E D 783   |
| 2735 GTA GTC GAA ATG AAC GAC AAC TTT GAA TTT GGA CTC TGC CCA TGT GAT GCC AAA CCC ATA 2794 |
| 784 V V E M N D N F E F G L C P C D A K P I 803   |
| 2795 GTA AGA GGG AAG TTC AAT ACA ACG CTG CTG AAC GGA CGG GCC TTC CAG ATG GTA TGC CCC 2854 |
| 804 V R G K F N T T L L N G P A F Q M V C P 823   |
| 2855 ATA GGA TGG ACA GGG ACT GTA AGC TGT ACG TCA TTC AAT ATG GAC ACC TTA GCC ACA ACT 2914 |
| 824 I G W T G T V S C T S F N M D T L A T T 843   |
| 2915 GTG GTA CGG ACA TAT AGA AGG TCT AAA CCA TTC CCT CAT AGG CAA GGC TGT ATC ACC CAA 2974 |
| 844 V V R T Y R R S K P F P H R Q G C I T Q 863   |
| 2975 AAG AAT CTG GGG GAG GAT CTC CAT AAC TGC ATC CTT GGA GGA AAT TGG ACT TGT GTG CCT 3034 |
| 864 K N L G E D L H N C I L G G N W T C V P 883   |
| 3035 GGA GAC CAA CTA CTA TAC AAA GGG GGC TCT ATT GAA TCT TGC AAG TGG TGT GGC TAT CAA 3094 |
| 884 G D Q L L Y K G G S I E S C K W C G Y Q 903   |
| 3095 TTT AAA GAG AGT GAG GGA CTA CCA CAC TAC CCC ATT GGC AAG TGT AAA TTG GAG AAC GAG 3154 |
| 904 F K E S E G L P H Y P I G K C K L E N E 923   |
| 3155 ACT GGT TAC AGG CTA GTA GAC AGT ACC TCT TGC ATT AGA GAA GGT GTG GCC ATA GTA CCA 3214 |
| 924 T G Y R L V D S T S C N R E G V A I V P 943   |
| 3215 CAA GGG ACA TTA AAG TGC AAG ATA GGA AAA ACA ACT GTA CAG GTC ATA GCT ATG GAT ACC 3274 |
| 944 Q G T L K C K I G K T T V Q V I A M D T 963   |
| 3275 AAA CTC GGA CCT ATG CCT TGC AGA CCA TAT GAA ATC ATA TCA AGT GAG GGG CCT GTA GAA 3334 |
| 964 K L G P M P C R P Y E I I S S E G P V E 983   |
| 3335 AAG ACA GCG TGT ACT TTC AAC TAC ACT AAG ACA TTA AAA AAT AAG TAT TTT GAG CCC AGA 3394 |
| 984 K T A C T F N Y T K T L K N K Y F E P R 1003  |

FIGURE 10-2

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|   |
|---|
| 3395 GAC AGC TAC TTT CAG CAA TAC ATG CTA AAA GGA GAG TAT CAA TAC TGG TTT GAC CTG GAG 3454 |
| 1004 D S Y F Q Q Y M L K G E Y Q Y W F D L E 1023   |
| 3455 GTG ACT GAC CAT CAC CGG GAT TAC TTC GCT GAG TCC ATA TTA GTG GTG GTA GTA GCC CTC 3514 |
| 1024 V T D H H R D Y F A E S I L V V V V A L 1043   |
| 3515 TTG GGT GGC AGA TAT GTA CTT TGG TTA CTG GTT ACA TAC ATG GTC TTA TCA GAA CAG AAG 3574 |
| 1044 L G R Y V L W L L V T Y M V L S E Q K 1063   |
| 3575 GCC TTA GGG ATT CAG TAT GGA TCA GGG GAA GTG GTG ATG ATG GGC AAC TTG CTA ACC CAT 3634 |
| 1064 A L G I Q Y G S G E V V M M G N L L T H 1083   |
| 3635 AAC AAT ATT GAA GTG GTG ACA TAC TTC TTG CTG CTG TAC CTA CTG CTG AGG GAG GAG AGC 3694 |
| 1084 N N I E V V T Y F L L L Y L L L R E E S 1103   |
| 3695 GTA AAG AAG TGG GTC TTA CTC TTA TAC CAC ATC TTA GTG GTA CAC CCA ATC AAA TCT GTA 3754 |
| 1104 V K K W V L L L Y H I L V V H P I K S V 1123   |
| 3755 ATT GTG ATC CTA CTG ATG ATT GGG GAT GTG GTA AAG GCC GAT TCA GGG GGC CAA GAG TAC 3814 |
| 1124 I V I L L M I G D V V K A D S G G Q E Y 1143   |
| 3815 TTG GGG AAA ATA GAC CTC TGT TTT ACA ACA GTA GTA CTA ATC GTC ATA GGT TTA ATC ATA 3874 |
| 1144 L G K I D L C F T T V V L I V I G L I I 1163   |
| 3875 GCC AGG CGT GAC CCA ACT ATA GTG CCA CTG GTA ACA ATA ATG GCA GCA CTG AGG GTC ACT 3934 |
| 1164 A R R D P T I V P L V T I M A A L R V T 1183   |
| 3935 GAA CTG ACC CAC CAG CCT GGA GTT GAC ATC GCT GTG GCG GTC ATG ACT ATA ACC CTA CTG 3994 |
| 1184 E L T H Q P G V D I A V A V M T I T L L 1203   |
| 3995 ATG GTT AGC TAT GTG ACA GAT TAT TTT AGA TAT AAA AAA TGG TTA CAG TGC ATT CTC AGC 4054 |
| 1204 M V S Y V T D Y F R Y K K W L Q C I L S 1223   |
| 4055 CTG GTA TCT GCG GTG TTC TTG ATA AGA AGC CTA ATA TAC CTA GGT AGA ATC GAG ATG CCA 4114 |
| 1224 L V S A V F L I R S L I Y L G R I E M P 1243   |
| 4115 GAG GTA ACT ATC CCA AAC TGG AGA CCA CTA ACT TTA ATA CTA TTA TAT TTG ATC TCA ACA 4174 |
| 1244 E V T I P N W R P L T L I L L Y L I S T 1263   |
| 4175 ACA ATT GTA ACG AGG TGG AAG GTT GAC GTG GCT GGC CTA TTG TTG CAA TGT GTG CCT ATC 4234 |
| 1264 T I V T R W K V D V A G L L L Q C V P I 1283   |
| 4235 TTA TTG CTG GTC ACA ACC TTG TGG GCC GAC TTC TTA ACC CTA ATA CTG ATC CTG CCT ACC 4294 |
| 1284 L L L V T T L W A D F L T L I L I L P T 1303   |
| 4295 TAT GAA TTG GTT AAA TTA TAC TAT CTG AAA ACT GTT AGG ACT GAT ATA GAA AGA AGT TGG 4354 |
| 1304 Y E L V K L Y Y L K T V R T D I E R S W 1323   |
| 4355 CTA GGG GGG ATA GAC TAT ACA AGA GTT GAC TCC ATC TAC GAC GTT GAT GAG AGT GGA GAG 4414 |
| 1324 L G G I D Y T R V D S I Y D V D E S G E 1343   |
| 4415 GGC GTA TAT CTT TTT CCA TCA AGG CAG AAA GCA CAG GGG AAT TTT TCT ATA CTC TTG CCC 4474 |
| 1344 G V Y L F P S R Q K A Q G N F S I L L P 1363   |
| 4475 CTT ATC AAA GCA ACA CTG ATA AGT TGC GTC AGC AGT AAA TGG CAG CTA ATA TAC ATG AGT 4534 |
| 1364 L I K A T L I S C V S S K W Q L I Y M S 1383   |
| 4535 TAC TTA ACT TTG GAC TTT ATG TAC TAC ATG CAC AGG AAA GTT ATA GAA GAG ATC TCA GGA 4594 |
| 1384 Y L T L D F M Y Y M H R K V I E E I S G 1403   |
| 4595 GGT ACC AAC ATA ATA TCC AGG TTA GTG GCA GCA CTC ATA GAG CTG AAC TGG TCC ATG GAA 4654 |
| 1404 G T N I I S R L V A A L I E L N W S M E 1423   |
| 4655 GAA GAG GAG AGC AAA GGC TTA AAG AAG TTT TAT CTA TTG TCT GGA AGG TTG AGA AAC CTA 4714 |
| 1424 E E S K G L K K F Y L L S G R L R N L 1443   |
| 4715 ATA ATA AAA CAT AAG GTA AGG AAT GAG ACC GTG GCT TCT TTG TAC GGG GAG GAG GAA GTC 4774 |
| 1444 I I K H K V R N E T V A S W Y G E E E V 1463   |
| 4775 TAC GGT ATG CCA AAG ATC ATG ACT ATA ATC AAG GCC AGT ACA CTG AGT AAG AGC AGG CAC 4834 |
| 1464 Y G M P K I M T I I K A S T L S K S R H 1483   |
| 4835 TGC ATA ATA TGC ACT GTA TGT GAG GCC CGA GAG TGG AAA GGT GGC ACC TGC CCA AAA TGT 4894 |
| 1484 C I I C T V C E G R E W K G G T C P K C 1503   |
| 4895 GGA CGC CAT GGG AAG CCG ATA ACG TGT GGG ATG TCG CTA GCA GAT TTT GAA GAA AGA CAC 4954 |
| 1504 G R H G K P I T C G M S L A D F E E R H 1523   |
| 4955 TAT AAA AGA ATC TTT ATA AGG GAA GGC AAC TTT GAG GGT ATG TGC AGC CGA TGC CAG GGA 5014 |
| 1524 Y K R I F I R E G N F E G M C S R C Q G 1543   |
| 5015 AAG CAT AGG AGG TTT GAA ATG GAC CGG GAA CCT AAG AGT GCC AGA TAC TGT GCT GAG TGT 5074 |
| 1544 K H R R F E M D R E P K S A R Y C A E C 1563   |
| 5075 AAT AGG CTG CAT CCT GCT GAG GAA GGT GAC TTT TGG GCA GAG TCG AGC ATG TTG GGC CTC 5134 |
| 1564 N R L H P A E E G D F W A E S S M L G L 1583   |

FIGURE 10-3

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|  |      |
|--|------|
| 5135 AAA ATC ACC TAC TTT GCG CTG ATG GAT GGA AAG GTG TAT GAT ATC ACA GAG TGG CCT CGA | 5194 |
| 1584 K I T Y F A L M D G K V Y D I T E W A G   | 1603 |
| 5195 TGC CAG CGT GTG GGA ATC TCC CCA GAT ACC CAC AGA GTC CCT TGT CAC ATC TCA TTT GGT | 5254 |
| 1604 C Q R V G I S P D T H R V P C H I S F G   | 1623 |
| 5255 TCA CGG ATG CCT TTC AGG CAG GAA TAC AAT GGC TTT GTA CAA TAT ACC GCT AGG GGG CAA | 5314 |
| 1624 S R M P F R Q E Y N G F V Q Y T A R G O   | 1643 |
| 5315 CTA TTT CTG AGA AAC TTG CCC GTA CTG GCA ACT AAA GTA AAA ATG CTC ATG GTA GGC AAC | 5374 |
| 1644 L F L R N L P V L A T K V K M L M V G N   | 1663 |
| 5375 CTT CGA GAA GAA ATT GTT AAT CTG GAA CAT CTT GGG TGG ATC CTA AGG GGG CCT GCC GTG | 5434 |
| 1664 L G E E I G N L E H L G W I L R G P A V   | 1683 |
| 5435 TGT AAG AAG ATC ACA GAG CAC GAA AAA TGC CAC ATT AAT ATA CTG GAT AAA CTA ACC GCA | 5494 |
| 1684 C K K I T E H E K C H I N I L D K L T A   | 1703 |
| 5495 TTT TTC GGG ATC ATG CCA AGG GGG ACT ACA CCC AGA GCC CCG GTG AGG TTC CCT ACG AGC | 5554 |
| 1704 F F G I M P R G T T P R A P V R F P T S   | 1723 |
| 5555 TTA CTA AAA GTG AGG AGG GGT CTG GAG ACT GCC TGG GCT TAC ACA CAC CAA GGC GGG ATA | 5614 |
| 1724 L L K V R R G L E T A W A Y T H Q G G I   | 1743 |
| 5615 AGT TCA GTC GAC CAT GTA ACC GCC GGA AAA GAT CTA CTG GTC TGT GAC AGC ATG GGA CGA | 5674 |
| 1744 S S V D H V T A G K D L L V C D S M G R   | 1763 |
| 5675 ACT AGA GTG GTT TGC CAA AGC AAC AAC AGG TTG ACC GAT GAG ACA GAG TAT GGC GTC AAG | 5734 |
| 1764 T R V V C Q S N N R L T D E T E Y G V K   | 1783 |
| 5735 ACT GAC TCA GGG TGC CCA GAC CGT GCC AGA TGT TAT GTG TTA AAT CCA GAG GCC GTT AAC | 5794 |
| 1784 T D S G C P D G A R C Y V L N P E A V N   | 1803 |
| 5795 ATA TCA GGA TCC AAA GGG GCA GTC GTT CAC CTC CAA AAG ACA GGT GGA GAA TTC ACG TGT | 5854 |
| 1804 I S G S K G A V V H L Q K T G G E F T C   | 1823 |
| 5855 GTC ACC GCA TCA GGC ACA CCG GCT TTC TTC GAC CTA AAA AAC TTG AAA GGA TGG TCA CGC | 5914 |
| 1824 V T A S G T P A F F D L K N L K G W S G   | 1843 |
| 5915 TTG CCT ATA TTT GAA GCC TCC AGC GGG AGG GTG GTT GGC AGA GTC AAA GTA GGG AAG AAT | 5974 |
| 1844 L P I F E A S S G R V V G R V K V G K N   | 1863 |
| 5975 GAA GAG TCT AAA CCT ACA AAA ATA ATG AGT GGA ATC CAG ACC GTC TCA AAA AAC AGA GCA | 6034 |
| 1864 E E S K P T K I M S G I Q T V S K N R A   | 1883 |
| 6035 GAC CTG ACC GAG ATG GTC AAG AAG ATA ACC AGC ATG AAC AGG GGA GAC TTC AAG CAG ATT | 6094 |
| 1884 D L T E M V K K I T S M N R G D F K Q I   | 1903 |
| 6095 ACT TTG GCA ACA GGG GCA GGC AAA ACC ACA GAA CTC CCA AAA GCA GTT ATA GAG GAG ATA | 6154 |
| 1904 T L A T G A G K T T E L P K A V I E E I   | 1923 |
| 6155 GGA AGA CAC AAG AGA GTA TTA GTT CTT ATA CCA TTA AGG GCA GCG GCA GAG TCA GTC TAC | 6214 |
| 1924 G R H K R V L V L I P L R A A A E S V Y   | 1943 |
| 6215 CAG TAT ATG AGA TTG AAA CAC CCA AGC ATC TCT TTT AAC CTA AGG ATA GGG GAC ATG AAA | 6274 |
| 1944 Q Y M R L K H P S I S F N L R I G D M K   | 1963 |
| 6275 GAG GGG GAC ATG GCA ACC GGG ATA ACC TAT GCA TCA TAC GGG TAC TTC TGC CAA ATG CCT | 6334 |
| 1964 E G D M A T G I T Y A S Y G Y F C Q M P   | 1983 |
| 6335 CAA CCA AAG CTC AGA GCT ATG GTA GAA TAC TCA TAC ATA TTC TTA GAT GAA TAC CAT     | 6394 |
| 1984 Q P K L R A A M V E Y S Y I F L D E Y H   | 2003 |
| 6395 TGT GCC ACT CCT GAA CAA CTG GCA ATT ATC GGG AAG ATC CAC AGA TTT TCA GAG AGT ATA | 6454 |
| 2004 C A T P E Q L A I I G K I H R F S E S I   | 2023 |
| 6455 AGG GTT GTC GCC ATG ACT GCC ACG CCA GCA GGG TCG GTG ACC ACA ACA GGT CAA AAG CAC | 6514 |
| 2024 R V V A M T A T P A G S V T T T G O K H   | 2043 |
| 6515 CCA ATA GAG GAA TTC ATA GCC CCC GAG GTA ATG AAA GGG GAG GAT CTT GGT AGT CAG TTC | 6574 |
| 2044 P I E E F I A P E V M K G E D L G S Q F   | 2063 |
| 6575 CTT GAT ATA GCA GGG TTA AAA ATA CCA GTG GAT GAG ATG AAA GGC AAT ATG TTG GTT TTT | 6634 |
| 2064 L D I A G L K I P V D E M K G N M L V F   | 2083 |
| 6635 GTA CCA ACG AGA AAC ATG GCA GTA GAG GTA GCA AAG AGG CTA AAA GCT AAG GGC TAT AAC | 6694 |
| 2084 V P T R N M A V E V A K K L K A K G Y N   | 2103 |
| 6695 TCT GGA TAC TAT TAC AGT GGA GAG GAT CCA GCC AAT CTG AGA GTT GTG ACA TCA CAA TCC | 6754 |
| 2104 S G Y Y Y S G E D P A N L R V V T S Q S   | 2123 |
| 6755 CCC TAT GTA ATC GTG GCT ACA AAT GCT ATT GAA TCA GGA GTG ACA CTA CCA GAT TTG GAC | 6814 |
| 2124 P Y V I V A T N A I E S G V T L P D L D   | 2143 |
| 6815 ACG GTT ATA GAC ACG GGG TTG AAA TGT GAA AAG AGG GTG AGG GTA TCA TCA AAG ATA CCC | 6874 |
| 2144 T V I D T G L K C E K R V R V S S K I P   | 2163 |

FIGURE 104

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6875 TTC ATC GTA ACA GGC CTT AAG AGC ATG GCC GTG ACT GTG GGT GAG CAG GCG CAG CGT AGG 6934  
 2164 F I V T G L K R M A V T V G E Q A Q R R 2183  
 6935 GGC AGA GTA GGT AGA GTG AAA CCC GGG AGG TAT TAT AGG AGC CAG GAA ACA GCA ACA GGG 6994  
 2184 G R V G R V K P G R Y Y R S Q E T A T G 2203  
 6995 TCA AAG GAC TAC CAC TAT GAC CTC TTG CAG GCA CAA AGA TAC GGG ATT GAG GAT GGA ATC 7054  
 2204 S K D Y H Y D L L Q A Q R Y G I E D G I 2223  
 7055 AAC GTG ACG AAA TCC TTT AGG GAG ATG AAT TAC GAT TGG AGC CTA TAC GAG GAG GAC AGC 7114  
 2224 N V T K S F R E M N Y D W S L Y E E D S 2243  
 7115 CTA CTA ATA ACC CAG CTG GAA ATA CTA AAT AAT CTA CTC ATC TCA GAA GAC TTG CCA GCC 7174  
 2244 L L I T Q L E I L N N L L I S E D L P A 2263  
 7175 GCT GTT AAG AAC ATA ATG GCC AGG ACT GAT CAC CCA GAG CCA ATC CAA CTT GCA TAC AAC 7234  
 2264 A V K N I M A R T D H P E P I Q L A Y N 2283  
 7235 AGC TAT GAA GTC CAG GTC CCG GTC CTG TTC CCA AAA ATA AGG AAT GGA GAA GTC ACA GAC 7294  
 2284 S Y E V Q V P V L F P K I R N G E V T D 2303  
 7295 ACC TAC GAA AAT TAC TCG TTT CTA AAT GCC AGA AAG TTA GGG GAG GAT GTG CCC GTG TAT 7354  
 2304 T Y E N Y S F L N A R K L G E D V P V Y 2323  
 7355 ATC TAC GCT ACT GAA GAT GAG GAT CTG GCA GTT GAC CTC TTA GGG CTA GAC TGG CCT GAT 7414  
 2324 I Y A T E D E D L A V D L L G L D W P D 2343  
 7415 CCT GGG AAC CAG CAG GTA GTG GAG ACT GGT AAA GCA CTG AAG CAA GTG ACC GGG TTG TCC 7474  
 2344 P G N Q Q V V E T G K A L K Q V T G L S 2363  
 7475 TCG GCT GAA AAT GCC CTA CTA GTG GCT TTA TTT GGG TAT GTG GGT TAC CAG GCT CTC TCA 7534  
 2364 S A E N A L L V A L F G Y V G Y Q A L S 2383  
 7535 AAG AGG CAT GTC CCA ATG ATA ACA GAC ATA TAT ACC ATC GAG GAC CAG AGA CTA GAA GAC 7594  
 2384 K R H V P M I T D I Y T I E D Q R L E D 2403  
 7595 ACC ACC CAC CTC CAG TAT GCA CCC AAC GCC ATA AAA ACC GAT GGG ACA GAG ACT GAA CTG 7654  
 2404 T T H L Q Y A P N A I K T D G T E T E L 2423  
 7655 AAA GAA CTG GCG TCG GGT GAC GTG GAA AAA ATC ATG GGA GCC ATT TCA GAT TAT GCA GCT 7714  
 2424 K E L A S G D V E K I M G A I S D Y A A 2443  
 7715 GGG GGA CTG GAG TTT GTT AAA TCC CAA GCA GAA AAG ATA AAA ACA GCT CCT TTG TTT AAA 7774  
 2444 G G L E F V K S Q A E K I K T A P L F K 2463  
 7775 GAA AAC GCA GAA GCC GCA AAA GGG TAT GTC CAA AAA TTC ATT GAC TCA TTA ATT GAA AAT 7834  
 2464 E N A E A A K G Y V Q K F I D S L I E N 2483  
 7835 AAA GAA GAA ATA ATC AGA TAT GGT TTG TGG GGA ACA CAC ACA GCA CTA TAC AAA AGC ATA 7894  
 2484 K E E I I R Y G L W G T H T A L Y K S I 2503  
 7895 GCT GCA AGA CTG GGG CAT GAA ACA GCG TTT GCC ACA CTA GTG TTA AAG TGG CTA GCT TTT 7954  
 2504 A A R L G H E T A F A T L V L K W L A F 2523  
 7955 GGA GGG GAA TCA GTG TCA GAC CAC GTC AAG CAG GCG GCA GTT GAT TTA GTG GTC TAT TAT 8014  
 2524 G G E S V S D H V K Q A A V D L V V Y Y 2543  
 8015 GTG ATG AAT AAG CCT TCC TTC CCA GGT GAC TCC GAG ACA CAG CAA GAA GGG AGG CGA TTC 8074  
 2544 V M N K P S F P G D S E T Q Q E G R R F 2563  
 8075 GTC GCA AGC CTC TTC ATC TCC GCA CTG GCA ACC TAC ACA TAC AAA ACT TGG AAT TAC CAC 8134  
 2564 V A S L F I S A L A T Y T Y K T W N Y H 2583  
 8135 AAT CTC TCT AAA GTG GTG GAA CCA GCC CTG GCT TAC CTC CCC TAT GCT ACC AGC GCA TTA 8194  
 2584 N L S K V V E P A L A Y L P Y A T S A L 2603  
 8195 AAA ATG TTC ACC CCA ACG CGG CTG GAG AGC GTG GTG ATA CTG AGC ACC ACG ATA TAT AAA 8254  
 2604 K M F T P T R L E S V V I L S T T I Y K 2623  
 8255 ACA TAC CTC TCT ATA AGG AAG GGG AAG AGT GAT GGA TTG CTG GGT ACG GGG ATA AGT GCA 8314  
 2624 T Y L S I R K G K S D G L L G T G I S A 2643  
 8315 GCC ATG GAA ATC CTG TCA CAA AAC CCA GTC TCG GTC GGT ATA TCT GTG ATG TTG GGG GTC 8374  
 2644 A M E I L S Q N P V S V G I S V M L G V 2663  
 8375 GGG GCA ATC GCT GCG CAC AAC GCT ATT GAG TCC AGT GAA CAG AAA AGG ACC CTA CTT ATG 8434  
 2664 G A I A A H N A I E S S E Q K R T L L M 2683  
 8435 AAG GTG TTT GTA AAG AAC TTC TTG GAT CAG GCT GCA ACA GAT GAG CTG GTA AAA GAA AAC 8494  
 2684 K V F V K N F L D Q A A T D E L V K E N 2703  
 8495 CCA GAA AAA ATT ATA ATG GCC TTA TTT GAA GCA GTC CAG ACA ATT GGT AAC CCC CTG AGA 8554  
 2704 P E K I I M A L F E A V Q T I G N P L R 2723  
 8555 CTA ATA TAC CAC CTG TAT GGG GTT TAC TAC AAA GGT TGG GAG GGC AAG GAA CTA TCT GAG 8614  
 2724 L I Y H L Y G V Y Y K C W E A K E L S E 2743

FIGURE 10-5

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8615 AGG ACA GCA GGC AGA AAC TTA TTC ACA TTG ATA ATG TTT GAA GCC TTC GAG TTA TTA CGG 8674  
 2744 R T A G R N L F T L I M F E A F E L L G 2763  
  
 8675 ATG GAC TCA CAA GGG AAA ATA AGG AAC CTG TCC GGA AAT TAC ATT TTG GAT TTG ATA TAC 8734  
 2764 M D S Q G K I R N L S G N Y I L D L I Y 2783  
  
 8735 GGC CTA CAC AAG CAA ATC AAC AGA GGG CTG AAG AAA ATG GTA CTG GGG TGG GCC CCT GCA 8794  
 2784 G L H K Q I N R G L K K M V L G W A P A 2803  
  
 8795 CCC TTT AGT TGT GAC TGG ACC CCT AGT GAC GAG AGG ATC AGA TTG CCA ACA GAC AAC TAT 8854  
 2804 P F S C D W T P S D E R I R L P T D N Y 2823  
  
 8855 TTG AGG GTA GAA ACC AGG TGC CCA TGT GGC TAT GAG ATG AAA GCT TTC AAA AAT GTA GGT 8914  
 2824 L R V E T R C P C G Y E M K A F K N V G 2843  
  
 8915 GGC AAA CTT ACC AAA GTG GAG GAG AGC GGG CCT TTC CTA TGT AGA AAC AGA CCT GGT AGG 8974  
 2844 G K L T K V E E S G P F L C R N R P G R 2863  
  
 8975 GGA CCA GTC AAC TAC AGA GTC ACC AAG TAT TAC GAT GAC AAC CTC AGA GAG ATA AAA CCA 9034  
 2864 G P V N Y R V T K Y Y D D D N L R E I K P 2883  
  
 9035 GTA GCA AAG TTG GAA GGA CAG GTA GAG CAC TAC TAC AAA GGG GTC ACA GCA AAA ATT GAC 9094  
 2884 V A K L E G Q V E H Y Y K G V T A K I D 2903  
  
 9095 TAC AGT AAA GGA AAA ATG CTC TTG GCC ACT GAC AAG TGG GAG GTG GAA CAT GGT GTC ATA 9154  
 2904 Y S K G K M L L A T D K W E V E H G V I 2923  
  
 9155 ACC AGG TTA GCT AAG AGA TAT ACT GGG GTC GGG TTC AAT GGT GCA TAC TTA GGT GAC GAG 9214  
 2924 T R L A K R Y T G V G F N G A Y L G D E 2943  
  
 9215 CCC AAT CAC CGT GCT CTA GTG GAG AGG GAC TGT GCA ACT ATA ACC AAA AAC ACA GTA CAG 9274  
 2944 P N H R A L V E R D C A T I T K N T V Q 2963  
  
 9275 TTT CTA AAA ATG AAG AGG GGG TGT GCG TTC ACC TAT GAC CTG ACC ATC TCC AAT CTG ACC 9334  
 2964 F L K M K K G C A F T Y D L T I S N L T 2983  
  
 9335 AGG CTC ATC GAA CTA GTA CAC AGG AAC AAT CTT GAA GAG AAG GAA ATA CCC ACC GCT ACG 9394  
 2984 R L I E L V H R N N L E E K E I P T A T 3003  
  
 9395 GTC ACC ACA TGG CTA GCT TAC ACC TTC GTG AAT GAA GAC GTA GGG ACT ATA AAA CCA GTA 9454  
 3004 V T T W L A Y T F V N E D V G T I K P V 3023  
  
 9455 CTA GGA GAG AGA GTA ATC CCC GAC CCT GTA GTT GAT ATC AAT TTA CAA CCA GAG GTG CAA 9514  
 3024 L G E R V I P D P V V D I N L Q P E V Q 3043  
  
 9515 GTG GAC ACG TCA GAG GTT GGG ATC ACA ATA ATT GGA AGG GAA ACC CTG ATG ACA ACG GGA 9574  
 3044 V D T S E V G I T I I G R E T L M T T G 3063  
  
 9575 GTG ACA CCT GTC TTG GAA AAA GTA GAG CCT GAC GCC AGC GAC AAC CAA AAC TCG GTG AAG 9634  
 3064 V T P V L E K V E P D A S D N Q N S V K 3083  
  
 9635 ATC GGG TTG GAT GAG GGT AAT TAC CCA GGG CCT GGA ATA CAG ACA CAT ACA CTA ACA GAA 9694  
 3084 I G L D E G N Y P G P G I Q T H T L T E 3103  
  
 9695 GAA ATA CAC AAC AGG GAT GCG AGG CCC TTC ATC ATG ATC CTG GGC TCA AGG AAT TCC ATA 9754  
 3104 E I H N R D A R P F I M I L G S R N S I 3123  
  
 9755 TCA AAT AGG GCA AAG ACT GCT AGA AAT ATA AAT CTG TAC ACA GGA AAT GAC CCC AGG GAA 9814  
 3124 S N R A K T A R N I N L Y T G N D P R E 3143  
  
 9815 ATA CGA GAC TTG ATG GCT GCA GGG CGC ATG TTA GTA GCA CTG AGG GAT GTC GAC CCT 9874  
 3144 I R D L M A A G R M L V V A L R D V D P 3163  
  
 9875 GAG CTG TCT GAA ATG GTC GAT TTC AAG GGG ACT TTT TTA GAT AGG GAG GCC CTG GAG GCT 9934  
 3164 E L S E M V D F K G T F L D R E A L E A 3183  
  
 9935 CTA AGT CTC GGG CAA CCT AAA CCG AAG CAG GTT ACC AAG GAA GCT GTT AGG AAT TTG ATA 9994  
 3184 L S L G Q P K P K Q V T K E A V R N L I 3203  
  
 9995 GAA CAG AAA AAA GAT GTG GAG ATC CCT AAC TGG TTT GCA TCA GAT GAC CCA GTA TTT CTG 10054  
 3204 E Q K K D V E I P N W F A S D D P V F L 3223  
  
 10055 GAA GTG GCC TTA AAA AAT GAT AAG TAC TAC TTA GTA GGA GAT GTT GGA GAG CTA AAA GAT 10114  
 3224 E V A L K N D K Y Y L V G D V G E L K D 3243  
  
 10115 CAA GCT AAA GCA CTT GGG GCC ACG GAT CAG ACA AGA ATT ATA AAG GAG GTA GCC TCA AGG 10174  
 3244 Q A K A L G A T D Q T R I I K E V G S R 3263  
  
 10175 ACG TAT GCC ATG AAG CTA TCT ACC TGG TTC CTC AAG GCA TCA AAC AAA CAG ATG AGT TTA 10234  
 3264 T Y A M K L S S W F L K A S N K O M S L 3283  
  
 10235 ACT CCA CTG TTT GAG GAA TTG TTG CTA CGG TGC CCA CCT GCA ACT AAG AGC AAT AAG GGG 10294  
 3284 T P L F E E L L R C P P A T K S N K G 3303  
  
 10295 CAC ATG GCA TCA GCT TAC CAA TTG GCA CAG GGT AAC TGG GAG CCC CTC GGT TGC GGG GTG 10354  
 3304 H M A S A Y Q L A Q G N W E P L G C G V 3323

FIGURE 10-6

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10355 CAC CTA GGT ACA ATA CCA GCC AGA AGG GTG AAG ATA CAC CCA TAT GAA CCT TAC CTG AAG 10414  
 3324 H L G T I P A R R V K I H P Y E A Y L K 3343

10415 TTG AAA GAT TTC ATA GAA GAA GAG AAG AAA CCT AGG GTT AAG GAT ACA GTA ATA AGA 10474  
 3344 L K D F I E E E E K K P R V K D T V I R 3363

10475 GAG CAC AAC AAA TGG ATA CTT AAA ATA AGG TTT CAA GGA AAC CTC AAC ACC AAG AAA 10534  
 3364 E H N K W I L K K I R F Q G N L N T K K 3383

10535 ATG CTC AAC CCG GGG AAA CTA TCT GAA CAG TTG GAC AGG GAG GGG CGC AAC AGG AAC ATC 10594  
 3384 M L N P G K L S E Q L D R E G R K R N I 3403

10595 TAC AAC CAC CAG ATT GGT ACT ATA ATG TCA AGT GCA CGC ATA AGG CTG GAG AAA TTG CCA 10654  
 3404 Y N H Q I G T I M S S A G I R L E K L P 3423

10655 ATA GTG AGG GCC CAA ACC GAC ACC AAA ACC TTT CAT GAG GCA ATA AGA GAT AAG ATA GAC 10714  
 3424 I V R A Q T D T K T F H E A I R D K I D 3443

10715 AAG AGT GAA AAC CGG CAA AAT CCA GAA TTG CAC AAC AAA TTG TTG GAG ATT TTC CAC ACG 10774  
 3444 K S E N R Q N P E L H N K L L E I F H T 3463

10775 ATA GCC CAA CCC ACC CTG AAA CAC ACC TAC GGT GAG GTG AGC TGG GAG CAA CTT GAG GCG 10834  
 3464 I A Q P T L K H T Y G E V T W E Q L E A 3483

10835 GGG ATA AAT AGA AAG GGG GCA GCA GGC TTC CTG GAG AAG AAC ATC GGA GAA GTA TTG 10894  
 3484 G I N R K G A A G F L E K K N I G E V L 3503

10895 GAT TCA GAA AAG CAC CTG GTA GAA CAA TTG GTC AGG GAT CTG AAG GCC GGG AGA AAG ATA 10954  
 3504 D S E K H L V E Q L V R D L K A G R K I 3523

10955 AAA TAT TAT GAA ACT GCA ATA CCA AAA AAT GAG AAG AGA GAT GTC AGT GAT GAC TGG CAG 11014  
 3524 K Y Y E T A I P K N E K R D V S D D W Q 3543

11015 GCA GGG GAC CTG GTG GTT GAG AAG AGG CCA AGA GTT ATC CAA TAC CCT GAA GCC AAG ACA 11074  
 3544 A G D L V V E K R P R V I Q Y P E A K T 3563

11075 AGG CTA GCC ATC ACT AAG GTC ATG TAT AAC TGG GTG AAA CAG CAG CCC GTT GTG ATT CCA 11134  
 3564 R L A I T K V M Y N W V K Q Q P V V I P 3583

11135 GGA TAT GAA GGA AAG ACC CCC TTG TTC AAC ATC TTT GAT AAA GTG AGA AAG GAA TGG GAC 11194  
 3584 G Y E G K T P L F N I F D K V R K E W D 3603

11195 TCG TTC AAT GAG CCA GTG GCC GTA AGT TTT GAC ACC AAA GCC TGG GAC ACT CAA GTG ACT 11254  
 3604 S F N E P V A V S F D T K A W D T Q V T 3623

11255 AGT AAG GAT CTG CAA CTT ATT GGA GAA ATC CAG AAA TAT TAC TAT AAG AAG GAG TGG CAC 11314  
 3624 S K D L Q L I G E I Q K Y Y K K E W H 3643

11315 AAG TTC ATT GAC ACC ATC ACC GAC CAC ATG ACA GAA GTA CCA GTT ATA ACA GCA GAT GGT 11374  
 3644 K F I D T I T D H M T E V P V I T A D G 3663

11375 GAA GTA TAT ATA AGA AAT GGG CAG AGA GGG AGC GGC CAG CCA GAC ACA AGT GCT CGC AAC 11434  
 3664 E V Y I R N G Q R G S G Q P D T S A G N 3683

11435 AGC ATG TTA AAT GTC CTG ACA ATG ATG TAC GGC TTC TGC GAA AGC ACA GGG GTA CCG TAC 11494  
 3684 S M L N V L T M M Y G F C E S T G V P Y 3703

11495 AAG AGT TTC AAC AGG GTG GCA AGG ATC CAC GTC TGT GGG GAT GAT GGC TTC TTA ATA ACT 11554  
 3704 K S F N R V A R I H V C G D D G F L I T 3723

11555 GAA AAA GGG TTA GGG CTG AAA TTT GCT AAC AAA GGG ATG CAG ATT CTT CAT GAA GCA GGC 11614  
 3724 E K G L G L K F A N K G M Q I L H E A G 3743

11615 AAA CCT CAG AAG ATA ACG GAA GGG GAA AAG ATG AAA GTT GCC TAT AGA TTT GAG GAT ATA 11674  
 3744 K P Q K I T E G E K M K V A Y R F E D I 3763

11675 GAG TTC TGT TCT CAT ACC CCA GTC CCT GTT AGG TGG TCC GAC AAC ACC AGT AGT CAC ATG 11734  
 3764 E F C S H T P V P V R W S D N T S S H M 3783

11735 GCC GGG AGA GAC ACC GCT GTG ATA CTA TCA AAG ATG GCA ACA AGA TTG GAT TCA AGT GGA 11794  
 3784 A G R D T A V I L S K M A T R L D S S G 3803

11795 GAG AGG GGT ACC ACA GCA TAT GAA AAA GCG GTA GCC TTC AGT TTC TTG CTG ATG TAT TCC 11854  
 3804 E R G T T A Y E K A V A F S F L L M Y S 3823

11855 TGG AAC CCG CTT GTT AGG AGG ATT TGC CTG TTG GTC CTT TCG CAA CAG CCA GAG ACA GAC 11914  
 3824 W N P L V R R I C L L V L S Q Q P E T D 3843

11915 CCA TCA AAA CAT GCC ACT TAT TAT TAC AAA GGT GAT CCA ATA GGG GCC TAT AAA GAT GTA 11974  
 3844 P S K H A T Y Y K G D P I G A Y K D V 3863

11975 ATA GGT CCG AAT CTA AGT GAA CTG AAG AGA ACA GGC TTT GAG AAA TTG GCA AAT CTA AAC 12034  
 3864 I G R N L S E L K R T G F E K L A N L N 3883

12035 CTA AGC CTG TCC ACC TTG GGG ATC TGG ACT AAG CAC ACA AGC AAA AGA ATA ATT CAG GAC 12094  
 3884 L S L S T L G I W T K H T S K R I I Q D 3903

FIGURE 10-7

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FIGURE 10-8

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**BVDV NADL (inf. clone) -> Genes**

DNA sequence 12578 b.p. gtatacgagaat ... ctaacagcccc linear

1 gtatacagaattaaaaaggcactcgatacgttggcaattaaaataattaggcttaggaaacaatccctc 80  
 81 tcagcgaaggccaaaaaggcttagccatgcctttagtaggactagcataatgagggggtagcaacagtggtagttcg 160  
 161 ttggatggctaagccctgagtcacagggttagtcgtcagtggtcgacgccttggaaataaggctcgcagatgccacgtgg 240  
 241 acgagggcatgccccaaagcacatcttaacctgagccccgggtcgcccaaggtttaaaccactgttacgata 320  
 321 cagcctgatagggtgtcagagggccactgtttactaaaaatctgttatggcac ATG GAG TTG 394  
 1 M E L 3  
 395 ATC ACA AAT GAA CTT TTA TAC AAA ACA TAC AAA CAA AAA CCC GTC GGG GTG GAG GAA CCT 454  
 4 I T N E L L Y K T Y K Q K P V G V E E P 23  
 455 GTT TAT GAT CAG GCA GGT GAT CCC TTA TTT GGT GAA AGG GGA GCA GTC CAC CCT CAA TCG 514  
 24 V Y D Q A G D P L F G E R G A V H P Q S 43  
 515 ACG CTA AAG CTC CCA CAC AAG AGA GGG GAA CGC GAT GTT CCA ACC AAC TTG GCA TCC TTA 574  
 44 T L K L P H K R G E R D V P T N L A S L 63  
 575 CCA AAA AGA GGT GAC TGC AGG TCG GGT AAT AGC AGA GGA CCT GTG AGC GGG ATC TAC CTG 634  
 64 P K R G D C R S G N S R G P V S G I Y L 83  
 635 AAG CCA GGG CCA CTA TTT TAC CAG GAC TAT AAA GGT CCC GTC TAT CAC AGG GCC CCG CTG 694  
 84 K P G P L F Y Q D Y K G P V Y H R A P L 103  
 695 GAG CTC TTT GAG GAG GGA TCC ATG TGT GAA ACG ACT AAA CGG ATA GGG AGA GTA ACT GGA 754  
 104 E L F E E G S M C E T T K R I G R V T G 123  
 755 AGT GAC GGA AAG CTG TAC CAC ATT TAT GTG TGT ATA GAT GGA TGT ATA ATA ATA AAA AGT 814  
 124 S D G K L Y H I Y V C I D G C I I I K S 143  
 815 GCC ACG AGA AGT TAC CAA AGG GTG TTC AGG TGG GTC CAT AAT AGG CTT GAC TGC CCT CTA 874  
 144 A T R S Y Q R V F R W V H N R L D C P L 163  
 875 TGG GTC ACA ACT TGC TCA GAC ACG AAA GAA GAG GGA GCA ACA AAA AGG AAA ACA CAG AAA 934  
 164 W V T T C S D T K E E G A T K K K T Q K 183  
 935 CCC GAC AGA CTA GAA AGG GGG AAA ATG AAA ATA GTG CCC AAA GAA TCT GAA AAA GAC AGC 994  
 184 P D R L E R G K M K I V P K E S E K D S 203  
 995 AAA ACT AAA CCT CCG GAT GCT ACA ATA GTG GTG GAA GGA GTC AAA TAC CAG GTG AGG AAG 1054  
 204 K T K P P D A T I V V E G V K Y Q V R K 223  
 1055 AAG GGA AAA ACC AAG AGT AAA AAC ACT CAG GAC GGC TTG TAC CAT AAC AAA AAC AAA CCT 1114  
 224 K G K T K S K N T Q D G L Y H N K N K P 243  
 1115 CAG GAA TCA CGC AAG AAA CTG GAA AAA GCA TTG TTG GCG TGG GCA ATA ATA GCT ATA GTT 1174  
 244 Q E S R K K L E K A L L A W A I I I A I V 263  
 1175 TTG TTT CAA GTT ACA ATG GGA GAA AAC ATA ACA CAG TGG AAC CTA CAA GAT AAT GGG ACG 1234  
 264 L F Q V T M G E N I T Q W N L Q D N G T 283  
 1235 GAA GGG ATA CAA CGG GCA ATG TTC CAA AGG GGT GTG AAT AGA AGT TTA CAT GGA ATC TGG 1294  
 284 E G I Q R A M F Q R G V N R S L H G I W 303  
 1295 CCA GAG AAA ATC TGT ACT GGT GTC CCT TCC CAT CTA GCC ACC GAT ATA GAA CTA AAA ACA 1354  
 304 P E K I C T G V P S H L A T D I E L K T 323  
 1355 ATT CAT GGT ATG ATG GAT GCA AGT GAG AAG ACC AAC TAC ACG TGT TGC AGA CTT CAA CGC 1414  
 324 I H G M M D A S E K T N Y T C C C R L Q R 343  
 1415 CAT GAG TGG AAC AAG CAT GGT TGG TGC AAC TGG TAC AAT ATT GAA CCC TGG ATT CTA GTC 1474  
 344 H E W N K H G W C N W Y N I E P W I L V 363  
 1475 ATG AAT AGA ACC CAA GCC AAT CTC ACT GAG GGA CAA CCA CCA AGG GAG TGC GCA GTC ACT 1534  
 364 M N R T Q A N L T E G Q P P R E C A V T 383  
 1535 TGT AGG TAT GAT AGG CCT AGT GAC TTA AAC GTG GTA ACA CAA GCT AGA GAT AGC CCC ACA 1594  
 384 C R Y D R A S D L N V V T Q A R D S P T 403  
 1595 CCC TTA ACA GGT TGC AAG AAA GGA AAG AAC TTC TCC TTT GCA GGC ATA TTG ATG CGG GGC 1654  
 404 P L T G C K K G K N F S F A G I L M R G 423-  
 1655 CCC TGC AAC TTT GAA ATA GCT GCA AGT GAT GTA TTA TTC AAA GAA CAT GAA CGC ATT AGT 1714  
 424 P C N F E I A A S D V L F K H E R I S 443  
 1715 ATG TTC CAG GAT ACT ACT CTT TAC CTT GTT GAC GGG TTG ACC AAC TCC TTA GAA GGT GCC 1774  
 444 M F Q D T T L Y L V D G L T N S L E G A 463

FIGURE 11-1

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BVDV NADL (inf. clone) -&gt; Ge...s

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|      |   |      |
|------|---|------|
| 1775 | AGA CAA GGA ACC GCT AAA CTG ACA ACC TCG TTA GCC AAG CAG CTC CGG ATA CTA GGA AAA | 1834 |
| 464  | R Q G T A K L T T W L G K Q L G I L G K   | 483  |
| 1835 | AAG TTG GAA AAC AAG AGT AAG ACC TGG TTT GGA GCA TAC GCT GCT TCC CCT TAC TGT GAT | 1894 |
| 484  | K L E N K S K T W F G A Y A A S P Y C D   | 503  |
| 1895 | GTC GAT CGC AAA ATT CGC TAC ATA TGG TAT ACA AAA AAT TGC ACC CCT GCC TGC TTA CCC | 1954 |
| 504  | V D R K I G Y I W Y T K N C T P A C L P   | 523  |
| 1955 | AAG AAC ACA AAA ATT GTC GCC CCT GGG AAA TTT GAC ACC AAT GCA GAG GAC CGC AAG ATA | 2014 |
| 524  | K N T K I V G P G K F D T N A E D G K I   | 543  |
| 2015 | TTA CAT GAG ATG GGG GGT CAC TTG TCG GAG GTC CTA CTA CTT TCT TTA GTG GTG CTG TCC | 2074 |
| 544  | L H E M G G H L S E V L L S L V V L S   | 563  |
| 2075 | GAC TTC GCA CCG GAA ACA GCT AGT GTA ATG TAC CTA ATC CTA CAT TTT TCC ATC CCA CAA | 2134 |
| 564  | D F A P E T A S V M Y L I L H F S I P Q   | 583  |
| 2135 | AGT CAC GTT GAT GTA ATG GAT TGT GAT AAG ACC CAG TTG AAC CTC ACA GTG GAG CTG ACA | 2194 |
| 584  | S H V D V M D C D K T Q L N L T V E L T   | 603  |
| 2195 | ACA GCT GAA GTA ATA CCA GGG TCG GTC TGG AAT CTA GGC AAA TAT GTC TGT ATA AGA CCA | 2254 |
| 604  | T A E V I P G S V W N L G K Y V C I R P   | 623  |
| 2255 | AAT TGG TGG CCT TAT GAG ACA ACT GTA GTG TTG GCA TTT GAA GAG GTG AGC CAG GTG GTG | 2314 |
| 624  | N W W P Y E T T V V L A F E E V S Q V V   | 643  |
| 2315 | AAG TTA GTG TTG AGG GCA CTC AGA GAT TTA ACA CGC ATT TGG AAC GCT GCA ACA ACT ACT | 2374 |
| 644  | K L V L R A L R D L T R I W N A A T T T   | 663  |
| 2375 | GCT TTT TTA GTA TGC CTT GTT AAG ATA GTC AGG GGC CAG ATG GTA CAG GGC ATT CTG TGG | 2434 |
| 664  | A F L V C L V K I V R G Q M V Q G I L W   | 683  |
| 2435 | CTA CTA TTG ATA ACA GGG GTA CAA GGG CAC TTG GAT TGC AAA CCT GAA TTC TCG TAT GCC | 2494 |
| 684  | L L I T G V Q G H L D C K P E F S Y A   | 703  |
| 2495 | ATA GCA AAG GAC GAA AGA ATT GGT CAA CTG GGG GCT GAA GGC CTT ACC ACC ACT TGG AAG | 2554 |
| 704  | I A K D E R I G Q L G A E G L T T T T W K                                       | 723  |
| 2555 | GAA TAC TCA CCT GGA ATG AAG CTG GAA GAC ACA ATG GTC ATT GCT TGG TGC GAA GAT GGG | 2614 |
| 724  | E Y S P G M K L E D T M V I A W C E D G   | 743  |
| 2615 | AAG TTA ATG TAC CTC CAA AGA TGC ACG AGA GAA ACC AGG TAT CTC GCA ATC TTG CAT ACA | 2674 |
| 744  | K L M Y L Q R C T R E T R Y L A I L H T   | 763  |
| 2675 | AGA GCC TTG CCG ACC AGT GTG GTA TTC AAA AAA CTC TTT GAT GGG CGA AAG CAA GAG GAT | 2734 |
| 764  | R A L P T S V V F K K L F D G R K Q E D   | 783  |
| 2735 | GTA GTC GAA ATG AAC GAC AAC TTT GAA TTT GGA CTC TGC CCA TGT GAT GCC AAA CCC ATA | 2794 |
| 784  | V V E M N D N F E F G L C P C D A K P I   | 803  |
| 2795 | GTA AGA GGG AAG TTC AAT ACA ACG CTG CTG AAC GGA CGG GCC TTC CAG ATG GTA TGC CCC | 2854 |
| 804  | V R G K F N T T L L N G P A F Q M V C P   | 823  |
| 2855 | ATA GGA TGG ACA GGG ACT GTA AGC TGT ACG TCA TTC AAT ATG GAC ACC TTA GCC ACA ACT | 2914 |
| 824  | I G W T G T V S C T S F N M D T L A T T   | 843  |
| 2915 | GTG GTA CGG ACA TAT AGA AGG TCT AAA CCA TTC CCT CAT AGG CAA GGC TGT ATC ACC CAA | 2974 |
| 844  | V R T Y R R S K P F P H R Q G C I T Q   | 863  |
| 2975 | AAG AAT CTG GGG GAG GAT CTC CAT AAC TGC ATC CTT GGA GGA AAT TGG ACT TGT GTG CCT | 3034 |
| 864  | K N L G E D L H N C I L G G N W T C V P   | 883  |
| 3035 | GGA GAC CAA CTA CTA TAC AAA GGG GCC TCT ATT GAA TCT TGC AAG TGG TGT GGC TAT CAA | 3094 |
| 884  | G D Q L L Y K G G S I E S C K W C G Y Q   | 903  |
| 3095 | TTT AAA GAG AGT GAG GGA CTA CCA CAC TAC CCC ATT GGC AAG TGT AAA TTG GAG AAC GAG | 3154 |
| 904  | F K E S E G L P H Y P I G K C K L E N E   | 923  |
| 3155 | ACT GGT TAC AGG CTA GTA GAC AGT ACC TCT TGC AAT AGA GAA GGT GTG GCC ATA GTA CCA | 3214 |
| 924  | T G Y R L V D S T S C N R E G V A I V P   | 943  |
| 3215 | CAA CGG ACA TTA AAG TGC AAG ATA GGA AAA ACA ACT GTA CAG GTC ATA GCT ATG GAT ACC | 3274 |
| 944  | Q G T L K C K I G K T T V Q V I A M D T   | 963  |
| 3275 | AAA CTC GGA CCT ATG CCT TGC AGA CCA TAT GAA ATC ATA TCA AGT GAG GGG CCT GTA GAA | 3334 |
| 964  | K L G P M P C R P Y E I I S S E G P V E   | 983  |
| 3335 | AAG ACA CGG TGT ACT TTC AAC TAC ACT AAG ACA TTA AAA AAT AAG TAT TTT GAG CCC AGA | 3394 |
| 984  | K T A C T F N Y T K T L K N K Y F E P R   | 1003 |
| 3395 | GAC AGC TAC TTT CAG CAA TAC ATG CTA AAA GGA GAG TAT CAA TAC TGG TTT GAC CTG GAG | 3454 |
| 1004 | D S Y F Q Q Y M L K G E Y Q Y W F D L E   | 1023 |
| 3455 | GTG ACT GAC CAT CAC CGG GAT TAC TTC GCT GAG TCC ATA TTA GTG GTG GTA GTC GCC CTC | 3514 |
| 1024 | V T D H H R D Y F A E S I L V V V V A L   | 1043 |

FIGURE 11-2

BVDV NADL (inf. clone) -&gt; Ge... .

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|      |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |      |
|------|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|
| 3515 | TTC | GGT | GCC | AGA | TAT | GTA | CTT | TGG  | TTA | CTG | GTT | ACA | TAC | ATG | GTC | TTA | TCA | GAA | CAG | AAG  | 3574 |
| 1044 | L   | G   | G   | R   | Y   | V   | L   | W    | L   | L   | V   | T   | Y   | M   | V   | L   | S   | E   | Q   | K    | 1063 |
| 3575 | GCC | TTA | GGG | ATT | CAG | TAT | GGA | TCA  | GGG | GAA | GTG | GTG | ATG | ATG | GGC | AAC | TTG | CTA | ACC | CAT  | 3634 |
| 1064 | A   | L   | G   | I   | Q   | Y   | G   | S    | G   | E   | V   | V   | M   | M   | G   | N   | L   | L   | T   | H    | 1083 |
| 3635 | AAC | AAT | ATT | GAA | GTG | GTG | ACA | TAC  | TTC | TTG | CTG | CTG | TAC | CTA | CTG | CTG | AGG | GAG | GAG | AGC  | 3694 |
| 1084 | N   | N   | I   | E   | V   | V   | T   | Y    | F   | L   | L   | L   | Y   | L   | L   | L   | R   | E   | E   | S    | 1103 |
| 3695 | GTA | AAG | AAG | TGG | GTC | TTA | CTC | TTA  | TAC | CAC | ATC | TTA | GTG | GTA | CAC | CCA | ATC | AAA | TCT | GTA  | 3754 |
| 1104 | V   | K   | K   | W   | V   | L   | L   | L    | Y   | H   | I   | L   | V   | V   | H   | P   | I   | K   | S   | V    | 1123 |
| 3755 | ATT | GTG | ATC | CTA | CTG | ATG | ATT | GGG  | GAT | GTG | GTA | AAG | GCC | GAT | TCA | GGG | GCC | CAA | GAG | TAC  | 3814 |
| 1124 | I   | V   | I   | L   | L   | M   | I   | G    | D   | V   | V   | K   | A   | D   | S   | G   | G   | Q   | E   | Y    | 1143 |
| 3815 | TTG | GGG | AAA | ATA | GAC | CTC | TGT | TTT  | ACA | ACA | GTA | GTA | CTA | ATC | GTC | ATA | GGT | TTA | ATC | ATA  | 3874 |
| 1144 | L   | G   | K   | I   | D   | L   | C   | F    | T   | T   | V   | V   | L   | I   | V   | I   | G   | L   | I   | I    | 1163 |
| 3875 | GCC | AGG | CGT | GAC | CCA | ACT | ATA | GTG  | CCA | CTG | GTA | ACA | ATA | ATG | GCA | GCA | CTG | AGG | GTC | ACT  | 3934 |
| 1164 | A   | R   | R   | D   | P   | T   | I   | V    | P   | L   | V   | T   | I   | M   | A   | A   | L   | R   | V   | T    | 1183 |
| 3935 | GAA | CTG | ACC | CAC | CAG | CCT | GGA | GTT  | GAC | ATC | GCT | GTG | GCG | GTC | ATG | ACT | ATA | ACC | CTA | CTG  | 3994 |
| 1184 | E   | L   | T   | H   | Q   | P   | G   | V    | D   | I   | A   | V   | A   | V   | M   | T   | I   | T   | L   | L    | 1203 |
| 3995 | ATG | GTT | AGC | TAT | GTG | ACA | GAT | TAT  | TTT | AGA | TAT | AAA | AAA | TGG | TTA | CAG | TGC | ATT | CTC | AGC  | 4054 |
| 1204 | M   | V   | S   | Y   | V   | T   | D   | Y    | F   | R   | Y   | K   | K   | W   | L   | Q   | C   | I   | L   | S    | 1223 |
| 4055 | CTG | GTA | TCT | GCG | GTG | TTC | TTG | ATA  | AGA | AGC | CTA | ATA | TAC | CTA | GGT | AGA | ATC | GAG | ATG | CCA  | 4114 |
| 1224 | L   | V   | S   | A   | V   | F   | L   | I    | R   | S   | L   | I   | Y   | L   | G   | R   | I   | E   | M   | P    | 1243 |
| 4115 | GAG | GTA | ACT | ATC | CCA | AAC | TGG | AGA  | CCA | CTA | ACT | TTA | ATA | CTA | TTA | TAT | TGG | ATC | TCA | ACA  | 4174 |
| 1244 | E   | V   | T   | I   | P   | N   | W   | R    | P   | L   | T   | L   | I   | L   | L   | Y   | L   | I   | S   | T    | 1263 |
| 4175 | ACA | ATT | GTA | ACG | AGG | TGG | AAG | GTT  | GAC | GTG | GCT | GCG | CTA | TTG | TTG | CAA | TGT | GTG | CCT | ATC  | 4234 |
| 1264 | T   | I   | V   | T   | R   | W   | K   | V    | D   | V   | A   | G   | L   | L   | L   | Q   | C   | V   | P   | I    | 1283 |
| 4235 | TTA | TTG | CTG | GTC | ACA | ACC | TTG | TGG  | GCC | GAC | TTC | TTA | ACC | CTA | ATA | CTG | ATC | CTG | CCT | ACC  | 4294 |
| 1284 | L   | L   | L   | V   | T   | T   | L   | W    | A   | D   | F   | L   | T   | L   | I   | L   | I   | L   | P   | T    | 1303 |
| 4295 | TAT | GAA | TTG | GTT | AAA | TTA | TAC | TAT  | CTG | AAA | ACT | GTT | AGG | ACT | GAT | ATA | GAA | AGA | AGT | TGG  | 4354 |
| 1304 | Y   | E   | L   | V   | K   | L   | Y   | Y    | L   | K   | T   | V   | R   | T   | D   | I   | E   | R   | S   | W    | 1323 |
| 4355 | CTA | GGG | GGG | ATA | GAC | TAT | ACA | AGA  | GTT | GAC | TCC | ATC | TAC | GAC | GTT | GAT | GAG | AGT | GGA | GAG  | 4414 |
| 1324 | L   | G   | G   | I   | D   | Y   | T   | R    | V   | D   | S   | I   | Y   | D   | V   | D   | E   | S   | G   | E    | 1343 |
| 4415 | GCG | GTA | TAT | CTT | TTT | CCA | TCA | AGG  | CAG | AAA | GCA | CAG | GGG | AAT | TTT | TCT | ATA | CTC | TTG | CCC  | 4474 |
| 1344 | G   | V   | Y   | L   | F   | P   | S   | R    | Q   | K   | A   | Q   | G   | N   | F   | S   | I   | L   | L   | P    | 1363 |
| 4475 | CTT | ATC | AAA | GCA | ACA | CTG | ATA | AGT  | TGC | GTC | AGC | AGT | AAA | TGG | CAG | CTA | ATA | TAC | ATG | AGT  | 4534 |
| 1364 | L   | I   | K   | A   | T   | L   | I   | S    | C   | V   | S   | S   | K   | W   | Q   | L   | I   | Y   | M   | S    | 1383 |
| 4535 | TAC | TTA | ACT | TTG | GAC | TTT | ATG | TAC  | TAC | ATG | CAC | AGG | AAA | GTT | ATA | GAA | GAG | ATC | TCA | GGA  | 4594 |
| 1384 | Y   | L   | T   | L   | D   | F   | M   | Y    | Y   | M   | H   | R   | K   | V   | I   | E   | E   | I   | S   | G    | 1403 |
| 4595 | GGT | ACC | AAC | ATA | ATA | TCC | AGG | TTA  | GTG | GCA | GCA | CTC | ATA | GAG | CTG | AAC | TGG | TCC | ATG | GAA  | 4654 |
| 1404 | G   | T   | N   | I   | I   | S   | R   | L    | V   | A   | A   | L   | I   | E   | L   | N   | W   | S   | M   | E    | 1423 |
| 4655 | GAA | GAG | GAG | AGC | AAA | GGC | TTA | AAG  | AAG | TTT | ATA | CTA | TTG | TCT | GGA | AGG | TTG | AGA | AAC | CTA  | 4714 |
| 1424 | E   | E   | E   | S   | K   | G   | L   | K    | K   | F   | Y   | L   | L   | S   | G   | R   | L   | R   | N   | L    | 1443 |
| 4715 | ATA | ATA | AAA | CAT | AAG | GTA | AGG | AAAT | GAG | ACC | GTG | GCT | TCT | TGG | TAC | GGG | GAG | GAG | GAA | GTC  | 4774 |
| 1444 | I   | I   | K   | H   | K   | V   | R   | N    | E   | T   | V   | A   | S   | W   | Y   | G   | E   | E   | V   | 1463 |      |
| 4775 | TAC | GGT | ATG | CCA | AAG | ATC | ATG | ACT  | ATA | ATC | AAG | GCC | AGT | ACA | CTG | AGT | AAG | AGC | AGG | CAC  | 4834 |
| 1464 | Y   | G   | M   | P   | K   | I   | M   | T    | I   | I   | K   | A   | S   | T   | L   | S   | K   | S   | R   | H    | 1483 |
| 4835 | TGC | ATA | ATA | TGC | ACT | GTA | TGT | GAG  | GGC | CGA | GAG | TGG | AAA | GGT | GGC | ACC | TGC | CCA | AAA | TGT  | 4894 |
| 1484 | C   | I   | I   | C   | T   | V   | C   | E    | G   | R   | E   | W   | K   | G   | G   | T   | C   | P   | K   | C    | 1503 |
| 4895 | GGA | CGC | CAT | GGG | AAG | CCG | ATA | ACG  | TGT | GGG | ATG | TCG | CTA | GCA | GAT | TTT | GAA | GAA | AGA | CAC  | 4954 |
| 1504 | G   | R   | H   | G   | K   | P   | I   | T    | C   | G   | M   | S   | L   | A   | D   | F   | E   | E   | R   | H    | 1523 |
| 4955 | TAT | AAA | AGA | ATC | TTT | ATA | AGG | GAA  | GGC | AAC | TTT | GAG | GGT | ATG | TGC | AGC | CGA | TGC | CAG | GGA  | 5014 |
| 1524 | Y   | K   | R   | I   | F   | I   | R   | E    | G   | N   | F   | E   | G   | M   | C   | S   | R   | C   | Q   | G    | 1543 |
| 5015 | AAG | CAT | AGG | AGG | TTT | GAA | ATG | GAC  | CGG | GAA | CCT | AAG | AGT | GCC | AGA | TAC | TGT | GCT | GAG | TGT  | 5074 |
| 1544 | K   | H   | R   | R   | F   | E   | M   | D    | R   | E   | P   | K   | S   | A   | R   | Y   | C   | A   | E   | C    | 1563 |
| 5075 | AAT | AGG | CTG | CAT | CCT | GCT | GAG | GAA  | GGT | GAC | TTT | TGG | GCA | GAG | TCG | AGC | ATG | TTG | GGC | CTC  | 5134 |
| 1564 | N   | R   | L   | H   | P   | A   | E   | E    | G   | D   | F   | W   | A   | E   | S   | S   | M   | L   | G   | L    | 1583 |
| 5135 | AAA | ATC | ACC | TAC | TTT | GCG | CTG | ATG  | GAT | GGA | AAG | GTG | TAT | GAT | ATC | ACA | GAG | TGG | GCT | GGA  | 5194 |
| 1584 | K   | I   | T   | Y   | F   | A   | L   | M    | D   | G   | K   | V   | Y   | D   | I   | T   | E   | W   | A   | G    | 1603 |
| 5195 | TGC | CAG | CGT | GTG | GGA | ATC | TCC | CCA  | GAT | ACC | CAC | AGA | GTC | CCT | TGT | CAC | ATC | TCA | TTT | GGT  | 5254 |
| 1604 | C   | O   | R   | V   | G   | I   | S   | P    | D   | T   | H   | R   | V   | P   | C   | H   | I   | S   | F   | G    | 1623 |

FIGURE 11-3

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BVDV NADL (inf. clone) -&gt; G.

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|  |      |
|--|------|
| 5255 TCA CGG ATG CCT TTC AGG CAG GAA TAC GGC TTT GTA CAA TAT ACC GCT AGG GGG CAA         | 5314 |
| 1624 S R M P F R Q E Y N G F V Q Y T A R G Q   | 1643 |
| 5315 CTA TTT CTG AGA AAC TTG CCC GTA CTG GCA ACT AAA GTA AAA ATG CTC ATG GTA GGC AAC     | 5374 |
| 1644 L F L R N L P V L A T K V K M L M V G N   | 1663 |
| 5375 CTT GGA GAA GAA ATT GGT ATT CTG GAA CAT CTT GGG TGG ATC CTA AGG GGG CCT GCC GTG     | 5434 |
| 1664 L G E E I G N L E H L L G W I L R G P A V   | 1683 |
| 5435 TGT AAG AAG ATC ACA GAG CAC GAA AAA TGC CAC ATT AAT ATA CTG GAT AAA CTA ACC GCA     | 5494 |
| 1684 C K K I T E H E K C H I N I L D K L T A   | 1703 |
| 5495 TTT TTC GGG ATC ATG CCA AGG GGG ACT ACA CCC AGA GCC CCG GTG AGG TTC CCT ACG AGC     | 5554 |
| 1704 F F G I M P R G T T P R A P V R F P T S   | 1723 |
| 5555 TTA CTA AAA GTG AGG AGG GGT CTG GAG ACT GCC TGG GCT TAC ACA CAC CAA GGC GGG ATA     | 5614 |
| 1724 L L K V R R G L E T A W A Y T H Q G G I   | 1743 |
| 5615 AGT TCA GTC GAC CAT GTA ACC GCC GGA AAA GAT CTA CTG GTC TGT GAC AGC ATG GGA CGA     | 5674 |
| 1744 S S V D H V T A G K D L L V C D S M G R   | 1763 |
| 5675 ACT AGA GTG GTT TGC CAA AGC AAC AAC AGG TTG ACC GAT GAG ACA GAG TAT GGC GTC AAG     | 5734 |
| 1764 T R V V C Q S N N R L T D E T E Y G V K   | 1783 |
| 5735 ACT GAC TCA GGG TGC CCA GAC GGT GCC AGA TGT TAT GTG TTA AAT CCA GAG GCC GTT AAC     | 5794 |
| 1784 T D S G C P D G A R C Y V L N P E A V N   | 1803 |
| 5795 ATA TCA GGA TCC AAA GGG GCA GTC GTT CAC CTC CAA AAG ACA GGT GGA GAA TTC ACG TGT     | 5854 |
| 1804 I S G S K G A V V H L Q K T G G E F T C   | 1823 |
| 5855 GTC ACC GCA TCA GGC ACA CCG GCT TTC TTC GAC CTA AAA AAC TTG AAA GGA TGG TCA GGC     | 5914 |
| 1824 V T A S G T P A F F D L K N L K G W S G   | 1843 |
| 5915 TTG CCT ATA TTT GAA GCC TCC AGC GGG AGG GTG GTT GGC AGA GTC AAA GTC GGG AAG AAT     | 5974 |
| 1844 L P I F E A S S G R V V G R V K V G K N   | 1863 |
| 5975 GAA GAG TCT AAA CCT ACA AAA ATA ATG AGT GGA ATC CAG ACC GTC TCA AAA AAC AGA GCA     | 6034 |
| 1864 E E S K P T K I M S G I Q T V S K N R A   | 1883 |
| 6035 GAC CTG ACC GAG ATG GTC AAG AAC ATA ACC AGC ATG AAC AGG GGA GAC TTC AAG CAG ATT     | 6094 |
| 1884 D L T E M V K K I T S M N R G D F K Q I   | 1903 |
| 6095 ACT TTG GCA ACA GGG GCA GGC AAA ACC ACA GAA CTC CCA AAA GCA GTT ATA GAG GAG ATA     | 6154 |
| 1904 T L A T G A G K T T E L P K A V I E E I   | 1923 |
| 6155 GGA AGA CAC AAG AGA GTA TTA GTT CTT ATA CCA TTA AGG GCA GCG GCA GAG TCA GTC TAC     | 6214 |
| 1924 G R H K R V L V L I P L R A A A E S V Y   | 1943 |
| 6215 CAG TAT ATG AGA TTG AAA CAC CCA AGC ATC TCT TTT AAC CTA AGG ATA GGG GAC ATG AAA     | 6274 |
| 1944 Q Y M R L K H P S I S F N L R I G D M K   | 1963 |
| 6275 GAG GGG GAC ATG GCA ACC GGG ATA ACC TAT GCA TCA TAC GGG TAC TTC TGC CAA ATG CCT     | 6334 |
| 1964 E G D M A T G I T Y A S Y G Y F C Q M P   | 1983 |
| 6335 CAA CCA AAG CTC AGA GCT GCT ATG GTA GAA TAC TCA TAC ATA TTC TTA GAT GAA TAC CAT     | 6394 |
| 1984 Q P K L R A A M V E Y S Y I F L D E Y H   | 2003 |
| 6395 TGT GCC ACT CCT GAA CAA CTG GCA ATT ATC GGG AAG ATC CAC AGA TTT TCA GAG AGT ATA     | 6454 |
| 2004 C A T P E Q L A I I G K I H R F S E S I   | 2023 |
| 6455 ACG GTT GTC GCC ATG ACT GCC ACG CCA GCA GGG TCG GTG ACC ACA ACA GGT CAA AAG CAC     | 6514 |
| 2024 R V V A M T A T P A G S V T T T G Q K H   | 2043 |
| 6515 CCA ATA GAG GAA TTC ATA GCC CCC GAG GTA ATG AAA GGG GAG GAT CTT GGT AGT CAG TTC     | 6574 |
| 2044 P I E E F I A P E V M K G E D L G S Q F   | 2063 |
| 6575 CTT GAT ATA GCA GGG TTA AAA ATA CCA GTG GAT GAG ATG AAA GGC AAT ATG TTG GTT TTT     | 6634 |
| 2064 L D I A G L K I P V D E M K G N M L V F   | 2083 |
| 6635 GTA CCA ACG AGA AAC ATG GCA GTA GAG GTA GCA AAG AAG CTA AAA GCT AAG GGC TAT AAC     | 6694 |
| 2084 V P T R N M A V E V A K K L K A K G Y N   | 2103 |
| 6695 TCT GGA TAC TAT TAC AGT GGA GAG GAT CCA GCC AAT CTG AGA GTT GTG ACA TCA CAA TCC     | 6754 |
| 2104 S G Y Y Y S G E D P A N L R V V T S Q S   | 2123 |
| 6755 CCC TAT GTA ATC GTG GCT ACA AAT GCT ATT GAA TCA GGA GTG ACA CTA CCA GAT TTG GAC     | 6814 |
| 2124 P Y V I V A T N A I E S G V T L P D L D   | 2143 |
| 6815 ACG GTT ATA GAC ACC GGG TTG AAA TGT GAA AAG AGG GTG AGG GTA TCA TCA AAG ATA CCC     | 6874 |
| 2144 T V I D T G L K C E K R V R V S S K I P   | 2163 |
| 6875 TTC ATC GTA ACA GCA GCC CTT AAG AGG ATG GCC GTG ACT GTG GGT GAG CAG CGG CAG CGT AGG | 6934 |
| 2164 F I V T G L K R M A V T V G E Q A O R R   | 2183 |
| 6935 GGC AGA GTA GGT AGA GTG AAA CCC CGG AGG TAT TAT AGG AGC CAG GAA ACA GCA ACA GGG     | 6994 |
| 2184 G R V G R V K P G R Y Y R S Q E T A T G   | 2203 |

FIGURE 11-4

BV DV NADL (inf. clone) -&gt; G+

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|      |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |     |      |      |
|------|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|
| 6995 | TCA | AAG | GAC | TAC | CAC | TAT | -GAC | CTC | TTC | CAG | GCA | CAA | AGA | TAC | GGG | ATT | GAG | GAT | GGA | ATC  | 7054 |
| 2204 | S   | K   | D   | Y   | H   | Y   | D    | L   | L   | Q   | A   | Q   | R   | Y   | G   | I   | E   | D   | G   | I    | 2223 |
| 7055 | AAC | GTG | ACG | AAA | TCC | TTT | AGG  | GAG | ATG | AAT | TAC | GAT | TGG | AGC | CTA | TAC | GAG | GAG | GAC | AGC  | 7114 |
| 2224 | N   | V   | T   | K   | S   | F   | R    | E   | M   | N   | Y   | D   | W   | S   | L   | Y   | E   | E   | D   | S    | 2243 |
| 7115 | CTA | CTA | ATA | ACC | CAG | CTG | GAA  | ATA | CTA | AAT | AAT | CTA | CTC | ATC | TCA | GAA | GAC | TTC | CCA | GCC  | 7174 |
| 2244 | L   | L   | I   | T   | Q   | L   | E    | I   | L   | N   | N   | L   | L   | I   | S   | E   | D   | L   | P   | A    | 2263 |
| 7175 | GCT | GTT | AAG | AAC | ATA | ATG | GCC  | AGG | ACT | GAT | CAC | CCA | GAG | CCA | ATC | CAA | CTT | GCA | TAC | AAC  | 7234 |
| 2264 | A   | V   | K   | N   | I   | M   | A    | R   | T   | D   | H   | P   | E   | P   | I   | Q   | L   | A   | Y   | N    | 2283 |
| 7235 | AGC | TAT | GAA | GTC | CAG | GTC | CCG  | GTC | CTG | TTC | CCA | AAA | ATA | AGG | AAT | GGA | GAA | GTC | ACA | GAC  | 7294 |
| 2284 | S   | Y   | E   | V   | Q   | V   | P    | V   | L   | F   | P   | K   | I   | R   | N   | G   | E   | V   | T   | D    | 2303 |
| 7295 | ACC | TAC | GAA | AAT | TAC | TCG | TTT  | CTA | AAT | GCC | AGA | AAG | TTA | GGG | GAG | GAT | GTG | CCC | GTG | TAT  | 7354 |
| 2304 | T   | Y   | E   | N   | Y   | S   | F    | L   | N   | A   | R   | K   | L   | G   | E   | D   | V   | P   | V   | Y    | 2323 |
| 7355 | ATC | TAC | GCT | ACT | GAA | GAT | GAG  | GAT | CTG | GCA | GTT | GAC | CTC | TTA | GGG | CTA | GAC | TGG | CCT | GAT  | 7414 |
| 2324 | I   | Y   | A   | T   | E   | D   | E    | D   | L   | A   | V   | D   | L   | L   | G   | L   | D   | W   | P   | D    | 2343 |
| 7415 | CCT | GGG | AAC | CAG | CAG | GTA | GTG  | GAG | ACT | GGT | AAA | GCA | CTG | AAG | CAA | GTG | ACC | GGG | TTG | TCC  | 7474 |
| 2344 | P   | G   | N   | Q   | Q   | V   | V    | E   | T   | G   | K   | A   | L   | K   | Q   | V   | T   | G   | L   | S    | 2363 |
| 7475 | TCG | GCT | GAA | AAT | GCC | CTA | CTA  | GTG | GCT | TTA | TTT | GGG | TAT | GTG | GGT | TAC | CAG | GCT | CTC | TCA  | 7534 |
| 2364 | S   | A   | E   | N   | A   | L   | L    | V   | A   | L   | F   | G   | Y   | V   | G   | Y   | Q   | A   | L   | S    | 2383 |
| 7535 | AAG | AGG | CAT | GTC | CCA | ATG | ATA  | ACA | GAC | ATA | TAT | ACC | ATC | GAG | GAC | CAG | AGA | CTA | GAA | GAC  | 7594 |
| 2384 | K   | R   | H   | V   | P   | M   | I    | T   | D   | I   | Y   | T   | I   | E   | D   | Q   | R   | L   | E   | D    | 2403 |
| 7595 | ACC | ACC | CAC | CTC | CAG | TAT | GCA  | CCC | AAC | GCC | ATA | AAA | ACC | GAT | GGG | ACA | GAG | ACT | GAA | CTG  | 7654 |
| 2404 | T   | T   | H   | L   | Q   | Y   | A    | P   | N   | A   | I   | K   | T   | D   | G   | T   | E   | T   | E   | L    | 2423 |
| 7655 | AAA | GAA | CTG | GCG | TCG | GGT | GAC  | GTG | GAA | AAA | ATC | ATG | GGG | GCA | ATT | TCA | GAT | TAT | GCA | GCT  | 7714 |
| 2424 | K   | E   | L   | A   | S   | G   | D    | V   | E   | K   | I   | M   | G   | A   | I   | S   | D   | Y   | A   | A    | 2443 |
| 7715 | GGG | GGA | CTG | GAG | TTT | GTT | AAA  | TCC | CAA | GCA | GAA | AAG | ATA | AAA | ACA | GCT | CCT | TTG | TTT | AAA  | 7774 |
| 2444 | G   | G   | L   | E   | F   | V   | K    | S   | Q   | A   | E   | K   | I   | K   | T   | A   | P   | L   | F   | K    | 2463 |
| 7775 | GAA | AAC | GCA | GAA | GCC | GCA | AAA  | GGG | TAT | GTC | CAA | AAA | TTC | ATT | GAC | TCA | TTA | ATT | GAA | AAT  | 7834 |
| 2464 | E   | N   | A   | E   | A   | A   | K    | G   | Y   | V   | Q   | K   | F   | I   | D   | S   | L   | I   | E   | N    | 2483 |
| 7835 | AAA | GAA | GAA | ATA | ATC | AGA | TAT  | GGT | TTG | TGG | GGG | ACA | CAC | ACA | GCA | CTA | TAC | AAA | AGC | ATA  | 7894 |
| 2484 | K   | E   | E   | I   | I   | R   | Y    | G   | L   | W   | G   | T   | H   | T   | A   | L   | Y   | K   | S   | I    | 2503 |
| 7895 | GCT | GCA | AGA | CTG | GGG | CAT | GAA  | ACA | GCG | TTT | GCC | ACA | CTA | GTG | TTA | AAG | TGG | CTA | GCT | TTT  | 7954 |
| 2504 | A   | A   | R   | L   | G   | H   | E    | T   | A   | F   | A   | T   | L   | V   | L   | K   | W   | L   | A   | F    | 2523 |
| 7955 | GGA | GGG | GAA | TCA | GTG | TCA | GAC  | CAC | GTC | AAG | CAG | GCG | GCA | GTT | GAT | TTA | GTG | GTC | TAT | TAT  | 8014 |
| 2524 | G   | G   | E   | S   | V   | S   | D    | H   | V   | K   | Q   | A   | A   | V   | D   | L   | V   | V   | Y   | Y    | 2543 |
| 8015 | GTG | ATG | AAT | AAG | CCT | TCC | TTC  | CCA | GGT | GAC | TCC | GAG | ACA | CAG | CAA | GAA | GGG | AGG | CGA | TTC  | 8074 |
| 2544 | V   | M   | N   | K   | P   | S   | F    | P   | G   | D   | S   | E   | T   | Q   | Q   | E   | G   | R   | R   | F    | 2563 |
| 8075 | GTC | GCA | AGC | CTG | TTC | ATC | TCC  | GCA | CTG | GCA | ACC | TAC | ACA | TAC | AAA | ACT | TGG | AAT | TAC | CAC  | 8134 |
| 2564 | V   | A   | S   | L   | F   | I   | S    | A   | L   | A   | T   | Y   | T   | Y   | K   | T   | W   | N   | Y   | H    | 2583 |
| 8135 | AAT | CTC | TCT | AAA | GTG | GTG | GAA  | CCA | GCC | CTG | GCT | TAC | CTC | CCC | TAT | GCT | ACC | AGC | GCA | TTA  | 8194 |
| 2584 | N   | L   | S   | K   | V   | V   | E    | P   | A   | L   | A   | Y   | L   | P   | Y   | A   | T   | S   | A   | L    | 2603 |
| 8195 | AAA | ATG | TTC | ACC | CCA | ACG | CGG  | CTG | GAG | AGC | GTG | GTG | ATA | CTG | AGC | ACC | ACG | ATA | TAT | AAA  | 8254 |
| 2604 | K   | M   | F   | T   | P   | T   | R    | L   | E   | S   | V   | V   | I   | L   | S   | T   | T   | I   | Y   | K    | 2623 |
| 8255 | ACA | TAC | CTC | TCT | ATA | AGG | AAG  | GGG | AAG | AGT | GAT | GGA | TTG | CTG | GGT | ACG | GGG | ATA | AGT | GCA  | 8314 |
| 2624 | T   | Y   | L   | S   | I   | R   | K    | G   | K   | S   | D   | G   | L   | L   | G   | T   | G   | I   | S   | A    | 2643 |
| 8315 | GCC | ATG | GAA | ATC | CTG | TCA | CAA  | AAC | CCA | GTA | TCG | GTA | GGT | ATA | TCT | GTG | ATG | TTG | GGG | GTA  | 8374 |
| 2644 | A   | M   | E   | I   | L   | S   | Q    | N   | P   | V   | S   | V   | G   | I   | S   | V   | M   | L   | G   | V    | 2663 |
| 8375 | GGG | GCA | ATC | GCT | GCG | CAC | AAC  | GCT | ATT | GAG | TCC | AGT | GAA | CAG | AAA | AGG | ACC | CTA | CTT | ATG  | 8434 |
| 2664 | G   | A   | I   | A   | A   | H   | N    | A   | I   | E   | S   | S   | E   | Q   | K   | R   | T   | L   | L   | M    | 2683 |
| 8435 | AAG | GTG | TTT | GTA | AAG | AAC | TTC  | TG  | GAT | CAG | GCT | GCA | ACA | GAT | GAG | CTG | GTA | AAA | GAA | AAC  | 8494 |
| 2684 | K   | V   | F   | V   | K   | N   | F    | L   | D   | Q   | A   | A   | T   | D   | E   | L   | V   | K   | E   | N    | 2703 |
| 8495 | CCA | GAA | AAA | ATT | ATA | ATG | GCC  | TTA | TTT | GAA | GCA | GTC | CAG | ACA | ATT | GGT | AAC | CCC | CTG | AGA  | 8554 |
| 2704 | P   | E   | K   | I   | I   | M   | A    | L   | F   | E   | A   | V   | Q   | T   | I   | G   | N   | P   | L   | R    | 2723 |
| 8555 | CTA | ATA | TAC | CAC | CTG | TAT | GGG  | GTT | TAC | AAA | GGT | TGG | GAG | GCC | AAG | GAA | CTA | TCT | GAG | 8614 |      |
| 2724 | L   | I   | Y   | H   | L   | Y   | G    | V   | Y   | Y   | K   | G   | W   | E   | A   | K   | E   | L   | S   | E    | 2743 |
| 8615 | AGG | ACA | GCA | GCC | AGA | AAC | TTA  | TTC | ACA | TTG | ATA | ATG | TTT | GAA | GCC | TTC | GAG | TTA | TTA | GGG  | 8674 |
| 2744 | R   | T   | A   | G   | R   | N   | L    | F   | T   | L   | I   | M   | F   | E   | A   | F   | E   | L   | L   | G    | 2763 |
| 8675 | ATG | GAC | TCA | CAA | GGG | AAA | ATA  | AGG | AAC | CTG | TCC | GGA | AAT | TAC | ATT | TTG | GAT | TTG | ATA | TAC  | 8734 |
| 2764 | M   | D   | S   | Q   | G   | K   | I    | R   | N   | L   | S   | G   | N   | Y   | I   | L   | D   | L   | I   | Y    | 2783 |

FIGURE 11-5

BVDV NADL (inf. clone) -&gt; Gt

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|       |     |     |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |       |       |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-------|-------|
| 8735  | GCG | CTA | CAC | AAG | CAA | ATC | AAC | AGA | GGG | CTG | AAG | AAA | ATG  | GTA | CTG | GGG | TGG | GCC | CCT | GCA   | 8794  |
| 2784  | G   | L   | H   | K   | Q   | I   | N   | R   | G   | L   | K   | K   | M    | V   | L   | G   | W   | A   | P   | A     | 2803  |
| 8795  | CCC | TTT | AGT | TGT | GAC | TGG | ACC | CCT | AGT | GAC | GAG | AGG | ATC  | AGA | TTG | CCA | ACA | GAC | AAC | TAT   | 8854  |
| 2804  | P   | F   | S   | C   | D   | W   | T   | P   | S   | D   | E   | R   | I    | R   | L   | P   | T   | D   | N   | Y     | 2823  |
| 8855  | TTG | AGG | GTA | GAA | ACC | AGG | TGC | CCA | TGT | GGC | TAT | GAG | ATG  | AAA | GCT | TTC | AAA | AAT | GTA | GGT   | 8914  |
| 2824  | L   | R   | V   | E   | T   | R   | C   | P   | C   | G   | Y   | E   | M    | K   | A   | F   | K   | N   | V   | G     | 2843  |
| 8915  | GGC | AAA | CTT | ACC | AAA | GTG | GAG | GAG | AGC | GGG | CCT | TTC | CTA  | TGT | AGA | AAC | AGA | CCT | GGT | AGG   | 8974  |
| 2844  | G   | K   | L   | T   | K   | V   | E   | S   | G   | P   | F   | L   | C    | R   | N   | R   | P   | G   | R   | 2863  |       |
| 8975  | GGA | CCA | GTC | AAC | TAC | AGA | GTC | ACC | AAG | TAT | TAC | GAT | GAC  | AAC | CTC | AGA | GAG | ATA | AAA | CCA   | 9034  |
| 2864  | G   | P   | V   | N   | Y   | R   | V   | T   | K   | Y   | Y   | D   | D    | N   | L   | R   | E   | I   | K   | P     | 2883  |
| 9035  | GTA | GCA | AAG | TTG | GAA | GGG | CAG | GTA | GAG | CAC | TAC | TAC | AAA  | GGG | GTC | ACA | GCA | AAA | ATT | GAC   | 9094  |
| 2884  | V   | A   | K   | L   | E   | G   | Q   | V   | E   | H   | Y   | Y   | K    | G   | V   | T   | A   | K   | I   | D     | 2903  |
| 9095  | TAC | AGT | AAA | GGA | AAA | ATG | CTC | TTG | GCC | ACT | GAC | AAG | TGG  | GAG | GTC | GAA | CAT | GGT | GTC | ATA   | 9154  |
| 2904  | Y   | S   | K   | G   | K   | M   | L   | L   | A   | T   | D   | K   | W    | E   | V   | E   | H   | G   | V   | I     | 2923  |
| 9155  | ACC | AGG | TTA | GCT | AAG | AGA | TAT | ACT | GGG | GTC | GGG | TTC | AAT  | GGT | GCA | TAC | TTA | GGT | GAC | GAG   | 9214  |
| 2924  | T   | R   | L   | A   | K   | R   | Y   | T   | G   | V   | G   | F   | N    | G   | A   | Y   | L   | G   | D   | E     | 2943  |
| 9215  | CCC | AAT | CAC | CGT | GCT | CTA | GTG | GAG | AGG | GAC | TGT | GCA | ACT  | ATA | ACC | AAA | AAC | ACA | GTA | CAG   | 9274  |
| 2944  | P   | N   | H   | R   | R   | A   | L   | V   | E   | R   | D   | C   | A    | T   | I   | T   | K   | N   | T   | V     | 2963  |
| 9275  | TTT | CTA | AAA | ATG | AAG | AAG | GGG | TGT | GCG | TTC | ACC | TAT | GAC  | CTG | ACC | ATC | TCC | AAT | CTG | ACC   | 9334  |
| 2964  | F   | L   | K   | M   | K   | K   | G   | C   | A   | F   | T   | Y   | D    | L   | T   | I   | S   | N   | L   | T     | 2983  |
| 9335  | AGG | CTC | ATC | GAA | CTA | GTA | CAC | AGG | AAC | AAT | CTT | GAA | GAG  | AAG | GAA | ATA | CCC | ACC | GCT | ACG   | 9394  |
| 2984  | R   | L   | I   | E   | L   | V   | H   | R   | N   | N   | L   | E   | E    | K   | E   | I   | P   | T   | A   | T     | 3003  |
| 9395  | GTC | ACC | ACA | TGG | CTA | GCT | TAC | ACC | TTC | GTG | AAT | GAA | GAC  | GTA | GGG | ACT | ATA | AAA | CCA | GTA   | 9454  |
| 3004  | V   | T   | T   | W   | L   | A   | Y   | T   | F   | V   | N   | E   | D    | V   | G   | T   | I   | K   | P   | V     | 3023  |
| 9455  | CTA | CGA | GAG | AGA | GTA | ATC | CCC | GAC | CCT | GTA | GTG | GAT | ATC  | AAT | TTA | CAA | CCA | GAG | GTG | CAA   | 9514  |
| 3024  | L   | G   | E   | R   | V   | I   | P   | D   | P   | V   | V   | D   | I    | N   | L   | Q   | P   | E   | V   | Q     | 3043  |
| 9515  | GTG | GAC | ACG | TCA | GAG | GTC | GGT | ATC | ACA | ATA | ATT | GGA | 'AGG | GAA | ACC | CTG | ATG | ACA | ACG | GGA   | 9574  |
| 3044  | V   | D   | T   | S   | E   | V   | G   | I   | T   | I   | I   | G   | R    | E   | T   | L   | M   | T   | T   | G     | 3063  |
| 9575  | GTG | ACA | CCT | GTC | TTG | GAA | AAA | GTA | GAG | CCT | GAC | GCC | AGC  | GAC | CAA | AAC | AC  | TCG | GTG | AAG   | 9634  |
| 3064  | V   | T   | P   | V   | L   | E   | K   | V   | E   | P   | D   | A   | S    | D   | N   | Q   | N   | S   | V   | K     | 3083  |
| 9635  | ATC | GGG | TTG | GAT | GAG | GGT | AAT | TAC | CCA | GGG | CCT | GGA | ATA  | CAG | ACA | CAT | ACA | ATA | ACA | GAA   | 9694  |
| 3084  | I   | G   | L   | D   | E   | G   | N   | Y   | P   | G   | P   | G   | I    | Q   | T   | H   | T   | L   | T   | E     | 3103  |
| 9695  | GAA | ATA | CAC | AAC | AGG | GAT | GCG | AGG | CCC | TTC | ATC | ATG | ATC  | CTG | GGC | TCA | AGG | AAT | TCC | ATA   | 9754  |
| 3104  | E   | I   | H   | N   | R   | D   | A   | R   | P   | F   | I   | M   | I    | L   | G   | S   | R   | N   | S   | I     | 3123  |
| 9755  | TCA | AAT | AGG | GCA | AAG | ACT | GCT | AGA | AAT | ATA | AAT | CTG | TAC  | ACA | GGA | AAT | GAC | CCC | AGG | GAA   | 9814  |
| 3124  | S   | N   | R   | A   | K   | T   | A   | R   | N   | I   | N   | L   | Y    | T   | G   | N   | D   | P   | R   | E     | 3143  |
| 9815  | ATA | CGA | GAC | TTG | ATG | GCT | GCA | GGG | CGC | ATG | TTA | GTA | GCA  | CTG | AGG | GAT | GTC | GAC | CCT | 9874  |       |
| 3144  | I   | R   | D   | L   | M   | A   | A   | G   | R   | M   | L   | V   | V    | A   | L   | R   | D   | V   | D   | P     | 3163  |
| 9875  | GAG | CTG | TCT | GAA | ATG | GTC | GAT | TTC | AAG | GGG | ACT | TTT | TTA  | GAT | AGG | GAG | GCC | CTG | GAG | GCT   | 9934  |
| 3164  | E   | L   | S   | E   | M   | V   | D   | F   | K   | G   | T   | F   | L    | D   | R   | E   | A   | L   | E   | A     | 3183  |
| 9935  | CTA | AGT | CTC | GGG | CAA | CCT | AAA | CCG | AAG | CAG | GTT | ACC | AAG  | GAA | GCT | GTT | AGG | AAT | TTG | ATA   | 9994  |
| 3184  | L   | S   | L   | G   | Q   | P   | K   | P   | K   | Q   | V   | T   | K    | E   | A   | V   | R   | N   | L   | I     | 3203  |
| 9995  | GAA | CAG | AAA | AAA | GAT | GTG | GAG | ATC | CCT | AAC | TCG | TTT | GCA  | TCA | GAT | GAC | CCA | GTA | TTT | CTG   | 10054 |
| 3204  | E   | Q   | K   | K   | D   | V   | E   | I   | P   | N   | W   | F   | A    | S   | D   | D   | P   | V   | F   | L     | 3223  |
| 10055 | GAA | GTG | GCC | TTA | AAA | AAT | GAT | AAG | TAC | TAC | TTA | GTA | GGA  | GAT | GTT | GGA | GAG | CTA | AAA | GAT   | 10114 |
| 3224  | E   | V   | A   | L   | K   | N   | D   | K   | Y   | Y   | L   | V   | G    | D   | V   | G   | E   | L   | K   | D     | 3243  |
| 10115 | CAA | GCT | AAA | GCA | CTT | GGG | GCC | ACG | GAT | CAG | ACA | AGA | ATT  | ATA | AAG | GAG | GTA | GGC | TCA | AGG   | 10174 |
| 3244  | Q   | A   | K   | A   | L   | G   | A   | T   | D   | Q   | T   | R   | I    | I   | K   | E   | V   | G   | S   | R     | 3263  |
| 10175 | ACG | TAT | GCC | ATG | AAG | CTA | TCT | AGC | TGG | TTC | CTC | AAG | GCA  | TCA | AAC | AAA | CAG | ATG | AGT | TTA   | 10234 |
| 3264  | T   | Y   | A   | M   | K   | L   | S   | S   | W   | F   | L   | K   | A    | S   | N   | K   | Q   | M   | S   | L     | 3283  |
| 10235 | ACT | CCA | CTG | TTT | GAG | GAA | TTG | TTG | CTA | CGG | TGC | CCA | CCT  | GCA | ACT | AAG | AGC | AAT | AAG | GGG   | 10294 |
| 3284  | T   | P   | L   | F   | E   | E   | L   | L   | R   | C   | P   | P   | A    | T   | K   | S   | N   | K   | G   | 3303  |       |
| 10295 | CAC | ATG | GCA | TCA | GCT | TAC | CAA | TTG | GCA | CAG | GGT | AAC | TGG  | GAG | CCC | CTC | GGT | TGC | GGG | GTG   | 10354 |
| 3304  | H   | M   | A   | S   | A   | Y   | Q   | L   | A   | Q   | G   | N   | W    | E   | P   | L   | G   | C   | G   | V     | 3323  |
| 10355 | CAC | CTA | GGT | ACA | ATA | CCA | CCC | AGA | AGG | GTG | AAG | ATA | CAC  | CCA | TAT | GAA | GCT | TAC | CTG | AAG   | 10414 |
| 3324  | H   | L   | G   | T   | I   | P   | A   | R   | R   | V   | K   | I   | H    | P   | Y   | E   | A   | Y   | L   | K     | 3343  |
| 10415 | TTG | AAA | GAT | TTC | ATA | GAA | GAA | GAA | GAG | AAA | CCT | AGG | GTT  | AAG | GAT | ACA | GTA | ATA | AGA | 10474 |       |
| 3344  | L   | K   | D   | F   | I   | E   | E   | E   | E   | K   | K   | P   | R    | V   | K   | D   | T   | V   | I   | R     | 3363  |

FIGURE 11-6

BVDV NADL (inf. clone) -&gt; G...s

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|       |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |      |       |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-------|
| 10475 | GAG | CAC | AAC | AAA | TGG | ATA | CTT | AAA | AAA  | ATA | AGG | TTT | CAA | GGA | AAC | CTC | AAC | ACC | AAG | AAA  | 10534 |
| 3364  | E   | H   | N   | K   | W   | I   | L   | K   | K    | I   | R   | F   | Q   | G   | N   | L   | N   | T   | K   | K    | 3383  |
| 10535 | ATG | CTC | AAC | CCG | GGG | AAA | CTA | TCT | GAA  | CAG | TTG | GAC | AGG | GAG | GGG | CGC | AAG | AGG | AAC | ATC  | 10594 |
| 3384  | M   | L   | N   | P   | G   | K   | L   | S   | E    | Q   | L   | D   | R   | E   | G   | R   | K   | R   | N   | I    | 3403  |
| 10595 | TAC | AAC | CAC | CAG | ATT | GGT | ACT | ATA | ATG  | TCA | AGT | GCA | GGC | ATA | AGG | CTG | GAG | AAA | TTG | CCA  | 10654 |
| 3404  | Y   | N   | H   | Q   | I   | G   | T   | I   | M    | S   | S   | A   | G   | I   | R   | L   | E   | K   | L   | P    | 3423  |
| 10655 | ATA | GTG | AGG | GCC | CAA | ACC | GAC | ACC | AAA  | ACC | TTT | CAT | GAG | GCA | ATA | AGA | GAT | AAG | ATA | GAC  | 10714 |
| 3424  | I   | V   | R   | A   | Q   | T   | D   | T   | K    | T   | F   | H   | E   | A   | I   | R   | D   | K   | I   | D    | 3443  |
| 10715 | AAG | AGT | GAA | AAC | CGG | CAA | AAT | CCA | GAA  | TTG | CAC | AAC | AAA | TTG | TTG | GAG | ATT | TTC | CAC | ACG  | 10774 |
| 3444  | K   | S   | E   | N   | R   | Q   | N   | P   | E    | L   | H   | N   | K   | L   | L   | E   | I   | F   | H   | T    | 3463  |
| 10775 | ATA | GCC | CAA | CCC | ACC | CTG | AAA | CAC | ACC  | TAC | GGT | GAG | GTG | ACG | TGG | GAG | CAA | CTT | GAG | GCG  | 10834 |
| 3464  | I   | A   | Q   | P   | T   | L   | K   | H   | T    | Y   | G   | E   | V   | T   | W   | E   | Q   | L   | E   | A    | 3483  |
| 10835 | GGG | ATA | AAT | AGA | AAG | GGG | GCA | GCA | GGC  | TTC | CTG | GAG | AAG | AAG | AAC | ATC | GGA | GAA | GTA | TTG  | 10894 |
| 3484  | G   | I   | N   | R   | K   | G   | A   | A   | G    | F   | L   | E   | K   | K   | N   | I   | G   | E   | V   | L    | 3503  |
| 10895 | GAT | TCA | GAA | AAG | CAC | CTG | GTA | GAA | CAA  | TTG | GTC | AGG | GAT | CTG | AAG | GCC | GGG | AGA | AAG | ATA  | 10954 |
| 3504  | D   | S   | E   | K   | H   | L   | V   | E   | Q    | L   | V   | R   | D   | L   | K   | A   | G   | R   | K   | I    | 3523  |
| 10955 | AAA | TAT | TAT | GAA | ACT | GCA | ATA | CCA | AAA  | AAT | GAG | AAG | AGA | GAT | GTC | AGT | GAT | GAC | TGG | CAG  | 11014 |
| 3524  | K   | Y   | Y   | E   | T   | A   | I   | P   | K    | N   | E   | K   | R   | D   | V   | S   | D   | D   | W   | Q    | 3543  |
| 11015 | GCA | GGG | GAC | CTG | GTG | GTT | GAG | AAG | AGG  | CCA | AGA | GTT | ATC | CAA | TAC | CCT | GAA | GCC | AAG | ACA  | 11074 |
| 3544  | A   | G   | D   | L   | V   | V   | E   | K   | R    | P   | R   | V   | I   | Q   | Y   | P   | E   | A   | K   | T    | 3563  |
| 11075 | AGG | CTA | GCC | ATC | ACT | AAG | GTC | ATG | TAT  | AAC | TTG | GTG | AAA | CAG | CAG | CCC | GTT | GTG | ATT | CCA  | 11134 |
| 3564  | R   | L   | A   | I   | T   | K   | V   | M   | Y    | N   | W   | V   | K   | Q   | Q   | P   | V   | V   | I   | P    | 3583  |
| 11135 | GGA | TAT | GAA | GGG | AAG | ACC | CCC | TTG | TTC  | AAC | ATC | TTT | GAT | AAA | GTG | AGA | AAG | GAA | TGG | GAC  | 11194 |
| 3584  | G   | Y   | E   | G   | K   | T   | P   | L   | F    | N   | I   | F   | D   | K   | V   | R   | K   | E   | W   | D    | 3603  |
| 11195 | TCG | TTC | AAT | GAG | CCA | GTG | GCC | GTA | AGT  | TTT | GAC | ACC | AAA | GCC | TGG | GAC | ACT | CAA | GTG | ACT  | 11254 |
| 3604  | S   | F   | N   | E   | P   | V   | A   | V   | S    | F   | D   | T   | K   | A   | W   | D   | T   | Q   | V   | T    | 3623  |
| 11255 | AGT | AAG | GAT | CTG | CAA | CIT | ATT | GGG | GAA  | ATC | CAG | AAA | TAT | TAC | TAT | AAG | AAG | GAG | TGG | CAC  | 11314 |
| 3624  | S   | K   | D   | L   | Q   | L   | I   | G   | E    | I   | Q   | K   | Y   | Y   | Y   | K   | K   | E   | W   | H    | 3643  |
| 11315 | AAG | TTC | ATT | GAC | ACC | ATC | ACC | GAC | CAC  | ATG | ACA | GAA | GTA | CCA | GTT | ATA | ACA | GCA | GAT | GGT  | 11374 |
| 3644  | K   | F   | I   | D   | T   | I   | T   | D   | H    | M   | T   | E   | V   | P   | V   | I   | T   | A   | D   | G    | 3663  |
| 11375 | GAA | GTA | TAT | ATA | AGA | AAT | GGG | CAG | AGA  | GGG | AGC | GCC | CAG | CCA | GAC | ACA | AGT | GCT | GGC | AAC  | 11434 |
| 3664  | E   | V   | Y   | I   | R   | N   | G   | Q   | R    | G   | S   | G   | Q   | P   | D   | T   | S   | A   | G   | N    | 3683  |
| 11435 | AGC | ATG | TTA | AAT | GTC | CTG | ACA | ATG | ATG  | TAC | GGC | TTC | TGC | GAA | AGC | ACA | GGG | GTA | CCG | TAC  | 11494 |
| 3684  | S   | M   | L   | N   | V   | L   | T   | M   | M    | Y   | G   | F   | C   | E   | S   | T   | G   | V   | P   | Y    | 3703  |
| 11495 | AAG | AGT | TTC | AAC | AGG | GTG | GCA | AGG | ATC  | CAC | GTC | TGT | GGG | GAT | GAT | GGC | TTC | TTA | ATA | ACT  | 11554 |
| 3704  | K   | S   | F   | N   | R   | V   | A   | R   | I    | H   | V   | C   | G   | D   | D   | G   | F   | L   | I   | T    | 3723  |
| 11555 | GAA | AAA | GGG | TTA | GGG | CTG | AAA | TTT | GCT  | AAC | AAA | GGG | ATG | CAG | ATT | CTT | CAT | GAA | GCA | GGC  | 11614 |
| 3724  | E   | K   | G   | L   | G   | L   | K   | F   | A    | N   | K   | G   | M   | Q   | I   | L   | H   | E   | A   | G    | 3743  |
| 11615 | AAA | CCT | CAG | AAG | ATA | ACG | GAA | GGG | GAA  | AAG | ATG | AAA | GTT | GCC | TAT | AGA | TTT | GAG | GAT | ATA  | 11674 |
| 3744  | K   | P   | Q   | K   | I   | T   | E   | G   | E    | K   | M   | K   | V   | A   | Y   | R   | F   | E   | D   | I    | 3763  |
| 11675 | GAG | TTC | TGT | TCT | CAT | ACC | CCA | GTC | CCT  | GTT | AGG | TGG | TCC | GAC | AAC | ACC | AGT | AGT | CAC | ATG  | 11734 |
| 3764  | E   | F   | C   | S   | H   | T   | P   | V   | P    | V   | R   | W   | S   | D   | N   | T   | S   | S   | H   | M    | 3783  |
| 11735 | GCC | GGG | AGA | GAC | ACC | GCT | GTG | ATA | CTA  | TCA | AAG | ATG | GCA | ACA | AGA | TTG | GAT | TCA | AGT | GGA  | 11794 |
| 3784  | A   | G   | R   | D   | T   | A   | V   | I   | L    | S   | K   | M   | A   | T   | R   | L   | D   | S   | S   | G    | 3803  |
| 11795 | GAG | AGG | GGT | ACC | ACA | GCA | TAT | GAA | AAA  | GCG | GTA | GCC | TTC | AGT | TTC | TTG | CTG | ATG | TAT | TCC  | 11854 |
| 3804  | E   | R   | G   | T   | T   | A   | Y   | E   | K    | A   | V   | A   | F   | S   | F   | L   | L   | M   | Y   | S    | 3823  |
| 11855 | TGG | AAC | CCG | CTT | GTT | AGG | AGG | ATT | TGC  | CTG | TTG | GTC | CTT | TCG | CAA | CAG | CCA | GAG | ACA | GAC  | 11914 |
| 3824  | W   | N   | P   | L   | V   | R   | R   | I   | C    | L   | L   | V   | L   | S   | Q   | Q   | P   | E   | T   | D    | 3843  |
| 11915 | CCA | TCA | AAA | CAT | GCC | ACT | TAT | TAT | TAC  | AAA | GGT | GAT | CCA | ATA | GGG | GCC | TAT | AAA | GAT | GTA  | 11974 |
| 3844  | P   | S   | K   | H   | A   | T   | Y   | Y   | K    | G   | D   | P   | I   | G   | A   | Y   | K   | D   | V   | 3863 |       |
| 11975 | ATA | GGT | CGG | AAT | CTA | AGT | GAA | CTG | AAAG | AGA | ACA | GGC | TTT | GAG | AAA | TTG | GCA | AAT | CTA | AAC  | 12034 |
| 3864  | I   | G   | R   | N   | L   | S   | E   | L   | K    | R   | T   | G   | F   | E   | K   | L   | A   | N   | L   | N    | 3883  |
| 12035 | CTA | AGC | CTG | TCC | ACG | TTG | GGG | ATC | TGG  | ACT | AAG | CAC | ACA | AGC | AAA | AGA | ATA | ATT | CAG | GAC  | 12094 |
| 3884  | L   | S   | L   | S   | T   | L   | G   | I   | W    | T   | K   | H   | T   | S   | K   | R   | I   | I   | Q   | D    | 3903  |
| 12095 | TGT | GTT | GCC | ATT | GGG | AAA | GAA | GAG | GCG  | AAC | TGG | CTA | GTT | AAC | GCC | GAC | AGG | CTG | ATA | TCC  | 12154 |
| 3904  | C   | V   | A   | I   | G   | K   | E   | E   | G    | N   | W   | L   | V   | N   | A   | D   | R   | L   | I   | S    | 3923  |
| 12155 | AGC | AAA | ACT | GGC | CAC | TTA | TAC | ATA | CCT  | GAT | AAA | GGC | TTT | ACA | TTA | CAA | GGG | AAG | CAT | TAT  | 12214 |
| 3924  | S   | K   | T   | G   | H   | L   | Y   | I   | P    | D   | K   | G   | F   | T   | L   | Q   | G   | K   | H   | Y    | 3943  |

FIGURE 11-7

BVDV NADL (inf. clone) -&gt; G...s

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12215 GAG CAA CTG CAG CTA AGA ACA GAG AAC CCG CTC ATG GGG GTT CGG ACT GAG AGA TAC 12274  
3944 E Q L Q L R T E T N P V M G V G T E R Y 3963

12275 AAG TTA GGT CCC ATA GTC AAT CTG CTG AGA AGG TTG AAA ATT CTG CTC ATG ACG GCC 12334  
3964 K L G P I V N L L R R L K I L L M T A 3983

12335 GTC CCC GTC AGC AGC TGA gacaaaaatgtataattgtaaataatccatgtacataatgttatataatataat 12408  
3984 V G V S S 3989

12409 agttgggaccgtccacctcaagaagacgacacgcggccaaacacgcacagctaaacagtagtcaagattatctacctaagat 12488

12489 aacactacattnaatgcacacagcacttagctgtatgaggatacgcccacgtctatagttggactagggaaagacctct 12568

12569 aacagcccc 12578

FIGURE 11-8

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## BVDV NADL cins- (inf. clone) -&gt; Genes

DNA sequence 12308 b.p. gtatacgagaat ... ctaacagccccc linear

|      |   |  |     |
|------|---|--|-----|
| 1    | gtatacgagaattaaaaaggcactcgatacgat   | ttggcaattaaaataataattaggcctagggaaacaaatccctc                         | 80  |
| 81   | tca   | cgaaaggccgaaaaaggctatgccttagttaggactacatgaggggggtagcaacagtggtagttcg  | 160 |
| 161  | ttggatggctta  | aggccctgagtcacagggtatgttcagtggttcacgccttggaaataaaaggctcgatgcccacgtgg | 240 |
| 241  | acgaggggcatgccccaaagcacatcttaac   | cgtgggggtcgcccaaggtaaaagcagttttaaccgactgttacgaata                    | 320 |
| 321  | cagcctgatagggtgctgcagaggcccactgtat  | tgtactaaaaatctctgtgtacatggcac ATG GAG TTG                            | 394 |
| 1    |   | M E L  | 3   |
| 395  | ATC ACA AAT GAA CTT TTA TAC AAA ACA TAC AAA CAA AAA CCC GTC GGG GTG GAG GAA CCT | 454  |     |
| 4    | I T N E L L Y K T Y K Q K P V G V E E P   | 23   |     |
| 455  | GTT TAT GAT CAG GCA GGT GAT CCC TTA TTT GGT GAA AGG GGA GCA GTC CAC CCT CAA TCG | 514  |     |
| 24   | V Y D Q A G D P L F G E R G A V H P Q S   | 43   |     |
| 515  | ACG CTA AAG CTC CCA CAC AAG AGA GGG GAA CGC GAT GTT CCA ACC AAC TTG GCA TCC TTA | 574  |     |
| 44   | T L K L P H K R G E R D V P T N L A S L   | 63   |     |
| 575  | CCA AAA AGA GGT GAC TGC AGG TCG GGT AAT AGC AGA GGA CCT GTG AGC GGG ATC TAC CTG | 634  |     |
| 64   | P K R G D C R S G N S R G P V S G I Y L   | 83   |     |
| 635  | AAG CCA GGG CCA CTA TTT TAC CAG GAC TAT AAA GGT CCC GTC TAT CAC AGG GCC CCG CTG | 694  |     |
| 84   | K P G P L F Y Q D Y K G P V Y H R A P L   | 103  |     |
| 695  | GAG CTC TTT GAG GAG GGA TCC ATG TGT GAA ACG ACT AAA CGG ATA GGG AGA GTA ACT GGA | 754  |     |
| 104  | E L F E E G S M C E T T K R I G R V T G   | 123  |     |
| 755  | AGT GAC GGA AAG CTG TAC CAC ATT TAT GTG TGT ATA GAT GGA TGT ATA ATA ATA AAA AGT | 814  |     |
| 124  | S D G K L Y H I Y V C I D G C I I I K S   | 143  |     |
| 815  | GCC ACG AGA AGT TAC CAA AGG GTG TTC AGG TGG GTC CAT AAT AGG CTT GAC TGC CCT CTA | 874  |     |
| 144  | A T R S Y Q R V F R W V H N R L D C P L   | 163  |     |
| 875  | TGG GTC ACA ACT TGC TCA GAC ACG AAA GAA GAG GGA GCA ACA AAA AGG AAA ACA CAG AAA | 934  |     |
| 164  | W V T T C S D T K E E G A T K K K T Q K   | 183  |     |
| 935  | CCC GAC AGA CTA GAA AGG GGG AAA ATG AAA ATA GTG CCC AAA GAA TCT GAA AAA GAC AGC | 994  |     |
| 184  | P D R L E R G K M K I V P K E S E K D S   | 203  |     |
| 995  | AAA ACT AAA CCT CCG GAT GCT ACA ATA GTG GTG GAA GGA GTC AAA TAC CAG GTG AGG AAG | 1054   |     |
| 204  | K T K P P D A T I V V E G V K Y Q V R K   | 223  |     |
| 1055 | AAG GCA AAA ACC AAG AGT AAA AAC ACT CAG GAC GGC TTG TAC CAT AAC AAA AAC AAA CCT | 1114   |     |
| 224  | K G K T K S K N T Q D G L Y H N K N K P   | 243  |     |
| 1115 | CAG GAA TCA CGC AAG AAA CTG GAA AAA GCA TTG TTG GCG TGG GCA ATA ATA GCT ATA GTT | 1174   |     |
| 244  | Q E S R K K L E K A L L A W A I I A I V   | 263  |     |
| 1175 | TTG TTT CAA GTT ACA ATG GGA GAA AAC ATA ACA CAG TGG AAC CTA CAA GAT AAT GGG ACG | 1234   |     |
| 264  | L F Q V T M G E N I T Q W N L Q D N G T   | 283  |     |
| 1235 | GAA CGG ATA CAA CGG GCA ATG TTC CAA AGG GGT GTG AAT AGA AGT TTA CAT GGA ATC TGG | 1294   |     |
| 284  | E G I Q R A M F Q R G V N R S L H G I W   | 303  |     |
| 1295 | CCA GAG AAA ATC TGT ACT GGT GTC CCT TCC CAT CTA GCC ACC GAT ATA GAA CTA AAA ACA | 1354   |     |
| 304  | P E K I C T G V P S H L A T D I E L K T   | 323  |     |
| 1355 | ATT CAT GGT ATG ATG GAT GCA AGT GAG AAC ACC AAC TAC ACC TGT TGC AGA CTT CAA CGC | 1414   |     |
| 324  | I H G M M D A S E K T N Y T C C R L Q R   | 343  |     |
| 1415 | CAT GAG TGG AAC AAG CAT GGT TGG AAC TGG TAC AAT ATT GAA CCC TGG ATT CTA GTC     | 1474   |     |
| 344  | H E W N K H G W C N W Y N I E P W I L V   | 363  |     |
| 1475 | ATG AAT AGA ACC CAA GGC AAT CTC ACT GAG GGA CAA CCA CCA AGG GAG TGC GCA GTC ACT | 1534   |     |
| 364  | M N R T Q A N L T E G Q P P R E C A V T   | 383  |     |
| 1535 | TGT AGG TAT GAT AGG GCT AGT GAC TTA AAC GTG GTC ACA CAA GCT AGA GAT AGC CCC ACA | 1594   |     |
| 384  | C R Y D R A S D L N V V T Q A R D S P T   | 403  |     |
| 1595 | CCC TTA ACA GGT TGC AAG AAA GGA AAG AAC TTC TCC TTT GCA GGC ATA TTG ATG CGG GGC | 1654   |     |
| 404  | P L T G C K K G K N F S F A G I L M R G   | 423  |     |
| 1655 | CCC TGC AAC TTT GAA ATA GCT GCA AGT GAT GTC TTA TTC AAA GAA CAT GAA CGC ATT AGT | 1714   |     |
| 424  | P C N F E I A A S D V L F K E H E R I S   | 443  |     |
| 1715 | ATG TTC CAG GAT ACT ACT CTT TAC CTT GTT GAC GGG TTG ACC AAC TCC TTA GAA GGT GCC | 1774   |     |
| 444  | M F Q D T T L Y L V D G L T N S L E G A   | 463  |     |

FIGURE 12-1

| BVDV NADL clns- (inf. clone) |   | Genes | 31/67 |         |            |      |   |
|------------------------------|---|-------|-------|---------|------------|------|---|
|                              |   |       |       | 4/21/99 | 5:45:24 PM | Page | 2 |
| 1775                         | AGA CAA GGA ACC GCT AAA CTG ACA ACC TCG TTA CGC AAG CAG CTC GGG ATA STA GGA AAA     |       |       | 1834    |            |      |   |
| 464                          | R Q G T A K L T T W L G K Q L G I L G K   |       |       | 483     |            |      |   |
| 1835                         | AAG TTG GAA AAC AAG AGT AAG ACG TGG TTT GGA GCA TAC GCT GCT TCC CCT TAC TGT GAT     |       |       | 1894    |            |      |   |
| 484                          | K L E N K S K T W F G A Y A A S P Y C D   |       |       | 503     |            |      |   |
| 1895                         | GTC GAT CGC AAA ATT GGC TAC ATA TGG TAT ACA AAA AAT TGC ACC CCT GCC TGC TTA CCC     |       |       | 1954    |            |      |   |
| 504                          | V D R K I G Y I W Y T K N C T P A C L P   |       |       | 523     |            |      |   |
| 1955                         | AAG AAC ACA AAA ATT GTC GGC CCT GGG AAA TTT GAC ACC AAT GCA GAG GAC CGC AAG ATA     |       |       | 2014    |            |      |   |
| 524                          | K N T K I V G P G K F D T N A E D G K I   |       |       | 543     |            |      |   |
| 2015                         | TTA CAT GAG ATG GGG GGT CAC TTG TCG GAG GTA CTA CTA CTT TCT TTA GTG GTG CTG TCC     |       |       | 2074    |            |      |   |
| 544                          | L H E M G G H L S E V L L L S L V V L S   |       |       | 563     |            |      |   |
| 2075                         | GAC TTC GCA CCG GAA ACA GCT AGT GTA ATG TAC CTA ATC CTA CAT TTT TCC ATC CCA CAA     |       |       | 2134    |            |      |   |
| 564                          | D F A P E T A S V M Y L I L H F S I P Q   |       |       | 583     |            |      |   |
| 2135                         | AGT CAC GTT GAT GTA ATG GAT TGT GAT AAG ACC CAG TTG AAC CTC ACA GTG GAG CTG ACA     |       |       | 2194    |            |      |   |
| 584                          | S H V D V M D C D K T Q L N L T V E L T   |       |       | 603     |            |      |   |
| 2195                         | ACA GCT GAA GTA ATA CCA GGG TCG GTC TGG AAT CTA GGC AAA TAT GTA TGT ATA AGA CCA     |       |       | 2254    |            |      |   |
| 604                          | T A E V I P G S V W N L G K Y V C I R P   |       |       | 623     |            |      |   |
| 2255                         | AAT TGG TGG CCT TAT GAG ACA ACT GTA GTG TTG GCA TTT GAA GAG GTG AGC CAG GTG GTG     |       |       | 2314    |            |      |   |
| 624                          | N W W P Y E T T V V L A F E E V S Q V V   |       |       | 643     |            |      |   |
| 2315                         | AAG TTA GTG TTG AGG GCA CTC AGA GAT TTA ACA CGC ATT TGG AAC GCT GCA ACA ACT ACT     |       |       | 2374    |            |      |   |
| 644                          | K L V L R A L R D L T R I W N A A T T T   |       |       | 663     |            |      |   |
| 2375                         | GCT TTT TTA GTA TGC CTT GTT AAG ATA GTC AGG GGC CAG ATG GTA CAG GGC ATT CTG TGG     |       |       | 2434    |            |      |   |
| 664                          | A F L V C L V K I V R G Q M V Q G I L W   |       |       | 683     |            |      |   |
| 2435                         | CTA CTA TTG ATA ACA GGG GTA CAA GGG CAC TTG GAT TGC AAA CCT GAA TTC TCG TAT GCC     |       |       | 2494    |            |      |   |
| 684                          | L L L I T G V Q G H L D C K P E F S Y A   |       |       | 703     |            |      |   |
| 2495                         | ATA GCA AAG GAC GAA AGA ATT GGT CAA CTG GGG GCT GAA GGC CTT ACC ACC ACT TGG AAG     |       |       | 2554    |            |      |   |
| 704                          | I A K D E R I G Q L G A E G L T T T W K   |       |       | 723     |            |      |   |
| 2555                         | GAA TAC TCA CCT GGA ATG AAG CTG GAA GAC ACA ATG GTC ATT GCT TGG TGC GAA GAT GGG     |       |       | 2614    |            |      |   |
| 724                          | E Y S P G M K L E D T M V I A W C E D G   |       |       | 743     |            |      |   |
| 2615                         | AAG TTA ATG TAC CTC CAA AGA TGC ACG AGA GAA ACC AGG TAT CTC GCA ATC TTG CAT ACA     |       |       | 2674    |            |      |   |
| 744                          | K L M Y L Q R C T R E T R Y L A I L H T   |       |       | 763     |            |      |   |
| 2675                         | AGA GCC TTG CCG ACC AGT GTG GTA TTC AAA AAA CTC TTT GAT GGG CGA AAG CAA GAG GAT     |       |       | 2734    |            |      |   |
| 764                          | R A L P T S V V F K K L F D G R K Q E D   |       |       | 783     |            |      |   |
| 2735                         | GTA GTC GAA ATG AAC GAC AAC TTT GAA TTT GGA CTC TGC CCA TGT GAT GCC AAA CCC ATA     |       |       | 2794    |            |      |   |
| 784                          | V V E M N D N F E F G L C P C D A K P I   |       |       | 803     |            |      |   |
| 2795                         | GTA AGA CGG AAG TTC AAT ACA ACG CTG CTG AAC GGA CCG GCC TTC CAG ATG GTA TGC CCC     |       |       | 2854    |            |      |   |
| 804                          | V R G K F N T T L L N G P A F Q M V C P   |       |       | 823     |            |      |   |
| 2855                         | ATA GGA TGG ACA GGG ACT GTA AGC TGT ACG TCA TTC AAT ATG GAC ACC TTA GCC ACA ACT     |       |       | 2914    |            |      |   |
| 824                          | I G W T G T V S C T S F N M D T L A T T   |       |       | 843     |            |      |   |
| 2915                         | GTG GTA CGG ACA TAT AGA AGG TCT AAA CCA TTC CCT CAT AGG CAA GGC TGT ATC ACC CAA     |       |       | 2974    |            |      |   |
| 844                          | V V R T Y R R S K P F P H R Q G C I T Q   |       |       | 863     |            |      |   |
| 2975                         | AAG AAT CTG GGG GAG GAT CTC CAT AAC TGC ATC CTT GGA GGA AAT TGG ACT TGT GTG CCT     |       |       | 3034    |            |      |   |
| 864                          | K N L G E D L H N C I L G G N W T C V P   |       |       | 883     |            |      |   |
| 3035                         | GGA GAC CAA CTA CTA TAC AAA GGG GGC TCT ATT GAA TCT TGC AAG TGG TGT GGC TAT CAA     |       |       | 3094    |            |      |   |
| 884                          | G D Q L L Y K G G S I E S C K W C G Y Q   |       |       | 903     |            |      |   |
| 3095                         | TTT AAA GAG AGT GAG GGA CTA CCA CAC TAC CCC ATT GGC AAG TGT AAA TTG GAG AAC GAG     |       |       | 3154    |            |      |   |
| 904                          | F K E S E G L P H Y P I G K C K L E N E   |       |       | 923     |            |      |   |
| 3155                         | ACT GGT TAC AGG CTA GTA GAC AGT ACC TCT TGC AAT AGA GAA GGT GTG GCC ATA GTA CCA     |       |       | 3214    |            |      |   |
| 924                          | T G Y R L V D S T S C N R E G V A I V P   |       |       | 943     |            |      |   |
| 3215                         | CAA GGG ACA TTA AAG TGC AAG ATA GGA AAA ACA ACT GTA CAG GTC ATA GCT ATG GAT ACC     |       |       | 3274    |            |      |   |
| 944                          | Q G T L K C K I G K T T V Q V I A M D T   |       |       | 963     |            |      |   |
| 3275                         | AAA CTC GGA CCT ATG CCT TGC AGA CCA TAT GAA ATC ATA TCA AGT GAG GGG CCT GTA GAA     |       |       | 3334    |            |      |   |
| 964                          | K L G P M P C R P Y E I I S S E G P V E   |       |       | 983     |            |      |   |
| 3335                         | AAG ACA GCG TGT ACT TTC AAC TAC ACT AAG ACA TTA AAA AAT AAG TAT TTT GAG CCC AGA     |       |       | 3394    |            |      |   |
| 984                          | K T A C T F N Y T K T L K N K Y F E P R   |       |       | 1003    |            |      |   |
| 3395                         | GAC AGC TAC TTT CAG CAA TAC ATG CTA AAA GGA GAG TAT CAA TAC TGG TTT GAC CTG GAG     |       |       | 3454    |            |      |   |
| 1004                         | D S Y F Q O Q Y M L K G E Y Q Y W F D L E   |       |       | 1023    |            |      |   |
| 3455                         | GTG ACT GAC CAT CAC CGG GAT TAC TTC GCT GAG TCC ATA TTA GTG GTG GTG GTC GCA CCC CTC |       |       | 3514    |            |      |   |
| 1024                         | V T D H H R D Y F A E S I L V V V V V A L   |       |       | 1043    |            |      |   |

FIGURE 12-2

| BVDV NADL cins- (inf. clone) | Genes   | 32/67 | 4/21/99 | 5:45:24 PM | Page 3 |
|------------------------------|---|-------|---------|------------|--------|
| - 3515                       | TTC GGT CGC AGA TAT GTC CTT TGG TTA CTG GTT ACA TAC ATG GTC TTA TCA GAA CAG AAG | 3574  |         |            |        |
| 1044 L                       | G G R Y V L W L L V T Y M V L S E Q K   | 1063  |         |            |        |
| 3575                         | GCC TTA GGG ATT CAG TAT GGA TCA GGG GAA GTG GTG ATG ATG CGC AAC TTG CTA ACC CAT | 3634  |         |            |        |
| 1064 A                       | L G I Q Y G S G E V V M M G N L L T H   | 1083  |         |            |        |
| 3635                         | AAC AAT ATT GAA GTG GTG ACA TAC TTC TTG CTG CTG TAC CTA CTG CTG AGG GAG GAG AGC | 3694  |         |            |        |
| 1084 N                       | N I E V V T Y F L L L Y L L R E E S   | 1103  |         |            |        |
| 3695                         | GTA AAG AAG TGG GTC TTA CTC TTA TAC CAC ATC TTA GTG GTA CAC CCA ATC AAA TCT GTA | 3754  |         |            |        |
| 1104 V                       | K K W V L L L Y H I L V V H P I K S V   | 1123  |         |            |        |
| 3755                         | ATT GTG ATC CTA CTG ATG ATT GGG GAT GTG GTA AAG GCC GAT TCA GGG GGC CAA GAG TAC | 3814  |         |            |        |
| 1124 I                       | V I L L M I G D V V K A D S G G Q E Y   | 1143  |         |            |        |
| 3815                         | TTC GGG AAA ATA GAC CTC TGT TTT ACA ACA GTA GTA CTA ATC GTC ATA GGT TTA ATC ATA | 3874  |         |            |        |
| 1144 L                       | G K I D L C F T T V V L I V I G L I I   | 1163  |         |            |        |
| 3875                         | GCC AGG CGT GAC CCA ACT ATA GTG CCA CTG GTA ACA ATA ATG GCA GCA CTG AGG GTC ACT | 3934  |         |            |        |
| 1164 A                       | R R D P T I V P L V T I M A A L R V T   | 1183  |         |            |        |
| 3935                         | GAA CTG ACC CAC CAG CCT GGA GTT GAC ATC GCT GTG GCG GTC ATG ACT ATA ACC CTA CTG | 3994  |         |            |        |
| 1184 E                       | L T H Q P G V D I A V A V M T I T L L   | 1203  |         |            |        |
| 3995                         | ATG GTT AGC TAT GTG ACA GAT TAT TTT AGA TAT AAA AAA TGG TTA CAG TGC ATT CTC AGC | 4054  |         |            |        |
| 1204 M                       | V S Y V T D Y F R Y K K W L Q C I L S   | 1223  |         |            |        |
| 4055                         | CTG GTA TCT GCG GTG TTC TTG ATA AGA AGC CTA ATA TAC CTA GGT AGA ATC GAG ATG CCA | 4114  |         |            |        |
| 1224 L                       | V S A V F L I R S L I Y L G R I E M P   | 1243  |         |            |        |
| 4115                         | GAG GTA ACT ATC CCA AAC TGG AGA CCA CTA ACT TTA ATA CTA TTA TAT TTG ATC TCA ACA | 4174  |         |            |        |
| 1244 E                       | V T I P N W R P L T L I L D Y L I S T   | 1263  |         |            |        |
| 4175                         | ACA ATT GTA ACG AGG TGG AAG GTT GAC GTG GCT GGC CTA TTG TTG CAA TGT GTG CCT ATC | 4234  |         |            |        |
| 1264 T                       | I V T R W K V D V A G L L L Q C V P I   | 1283  |         |            |        |
| 4235                         | TTA TTG CTG GTC ACA ACC TTG TGG GCC GAC TTC TTA ACC CTA ATA CTG ATC CTG CCT ACC | 4294  |         |            |        |
| 1284 L                       | L V T T L W A D F L T L I L I L P T   | 1303  |         |            |        |
| 4295                         | TAT GAA TTG GTT AAA TTA TAC TAT CTG AAA ACT GTT AGG ACT GAT ATA GAA AGA AGT TGG | 4354  |         |            |        |
| 1304 Y                       | E L V K L Y Y L K T V R T D I E R S W   | 1323  |         |            |        |
| 4355                         | CTA GGG GGG ATA GAC TAT ACA AGA GTT GAC TCC ATC TAC GAC GTT GAT GAG AGT GGA GAG | 4414  |         |            |        |
| 1324 L                       | G G I D Y T R V D S I Y D V D E S G E   | 1343  |         |            |        |
| 4415                         | GCG GTA TAT CTT TTT CCA TCA AGG CAG AAA GCA CAG GGG AAT TTT TCT ATA CTC TTG CCC | 4474  |         |            |        |
| 1344 G                       | V Y L F P S R Q K A Q G N F S I L L P   | 1363  |         |            |        |
| 4475                         | CTT ATC AAA GCA ACA CTG ATA AGT TGC GTC AGC AGT AAA TGG CAG CTA ATA TAC ATG AGT | 4534  |         |            |        |
| 1364 L                       | I K A T L I S C V S S K W Q L I Y M S   | 1383  |         |            |        |
| 4535                         | TAC TTA ACT TTG GAC TTT ATG TAC TAC ATG CAC AGG AAA GTT ATA GAA GAG ATC TCA GGA | 4594  |         |            |        |
| 1384 Y                       | L T D F M Y Y M H R K V I E E I S G   | 1403  |         |            |        |
| 4595                         | GGT ACC AAC ATA ATA TCC AGG TTA GTG GCA GCA CTC ATA GAG CTG AAC TGG TCC ATG GAA | 4654  |         |            |        |
| 1404 G                       | T N I I S R L V A A L I E L N W S M E   | 1423  |         |            |        |
| 4655                         | GAA GAG GAG AGC AAA GGC TTA AAG AAG TTT TAT CTA TTG TCT GGA AGG TTG AGA AAC CTA | 4714  |         |            |        |
| 1424 E                       | E S K G L K K F Y L L S G R L R N L   | 1443  |         |            |        |
| 4715                         | ATA ATA AAA CAT AAG GTA AGG AAT GAG ACC GTG GCT TCT TTG TAC GGG GAG GAG GAA GTC | 4774  |         |            |        |
| 1444 I                       | I K H K V R N E T V A S W Y G E E E V   | 1463  |         |            |        |
| 4775                         | TAC GGT ATG CCA AAG ATC ATG ACT ATA ATC AAG GCC AGT ACA CTG AGT AAG AGC AGG CAC | 4834  |         |            |        |
| 1464 Y                       | G M P K I M T I I K A S T L S K S R H   | 1483  |         |            |        |
| 4835                         | TGC ATA ATA TGC ACT GTA TGT GAG GCC CGA GAG TGG AAA GGT GGC ACC TGC CCA AAA TGT | 4894  |         |            |        |
| 1484 C                       | I I C T V C E G R E W K G G T C P K C   | 1503  |         |            |        |
| 4895                         | GGA CGC CAT GGG AAG CCG ATA ACG TGT GGG ATG TCG CTA GCA GAT TTT GAA GAA AGA CAC | 4954  |         |            |        |
| 1504 G                       | R H G K P I T C G M S L A D F E E R H   | 1523  |         |            |        |
| 4955                         | TAT AAA AGA ATC TTT ATA AGG GAA GCC AAC TTT GAG gggccc TTC AGG CAG GAA TAC AAT  | 5014  |         |            |        |
| 1524 Y                       | K R I F I R E G N F E F R Q E Y N   | 1541  |         |            |        |
| 5015                         | GCG TTT GTA CAA TAT ACC GCT AGG GGG CAA CTA TTT CTG AGA AAC TTG CCC GTC CTG GCA | 5074  |         |            |        |
| 1542 G                       | F V Q Y T A R G Q L F L R N L P V L A   | 1561  |         |            |        |
| 5075                         | ACT AAA GTA AAA ATG CTC ATG GTA GGC AAC CTT GGA GAA GAA ATT GGT AAT CTG GAA CAT | 5134  |         |            |        |
| 1562 T                       | K V K M L M V G N L G E E I G N L E H   | 1581  |         |            |        |
| 5135                         | CTT GGG TGG ATC CTA AGG GGG CCT GCC GTG TGT AAG AAG ATC ACA GAG CAC GAA AAA TGC | 5194  |         |            |        |
| 1582 L                       | G W I L R G P A V C K K I T E H E K C   | 1601  |         |            |        |
| 5195                         | CAC ATT AAT ATA CTG GAT AAA CTA ACC GCA TTT TTC GGG ATC ATG CCA AGG GGG ACT ACA | 5254  |         |            |        |
| 1602 H                       | I N I L D K L T A F F G I M P R G T T   | 1621  |         |            |        |

FIGURE 12-3

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BVDV NADL cins- (inf. clone) Genes

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|  |      |
|--|------|
| 5255 CCC AGA GCC CCG GTG AGG TTC CCT ACG AGC TTA CTA AAA GTG AGG AGG GGT CTG GAG ACT     | 5314 |
| 1622 P R A P V R F P T S L L K V R R G L E T   | 1641 |
| 5315 GCC TGG GCT TAC ACA CAC CAA GCC GGG ATA AGT TCA GTC GAC CAT GTA ACC GCC GGA AAA     | 5374 |
| 1642 A W A Y T H Q G G I S S V D H V T A G K   | 1661 |
| 5375 GAT CTA CTG GTC TGT GAC AGC ATG GGA CGA ACT AGA GTG GTT TGC CAA AGC AAC AAC AGG     | 5434 |
| 1662 D L L V C D S M G R T R V V C Q S N N R   | 1681 |
| 5435 TTG ACC GAT GAG ACA GAG TAT GGC GTC AAG ACT GAC TCA GGG TGC CCA GAC GGT GCC AGA     | 5494 |
| 1682 L T D E T E Y G V K T D S G C P D G A R   | 1701 |
| 5495 TGT TAT GTG TTA AAT CCA GAG GCC GTT AAC ATA TCA GGA TCC AAA GGG GCA GTC GTT CAC     | 5554 |
| 1702 C Y V L N P E A V N I S G S K G A V V H   | 1721 |
| 5555 CTC CAA AAG ACA GGT GGA GAA TTC ACG TGT GTC ACC GCA TCA GGC ACA CCG GCT TTC TTC     | 5614 |
| 1722 L Q K T G G E F T C V T A S G T P A F F   | 1741 |
| 5615 GAC CTA AAA AAC TTG AAA GGA TGG TCA GGC TTG CCT ATA TTT GAA GCC TCC AGC GGG AGG     | 5674 |
| 1742 D L K N L K G W S G L P I F E A S S G R   | 1761 |
| 5675 GTG GTT GGC AGA GTC AAA GTA GGG AAG AAT GAA GAG TCT AAA CCT ACA AAA ATA ATG AGT     | 5734 |
| 1762 V V G R V K V G K N E E S K P T K I M S   | 1781 |
| 5735 GGA ATC CAG ACC GTC TCA AAA AAC AGA GCA GAC CTG ACC GAG ATG GTC AAG AAG ATA ACC     | 5794 |
| 1782 G I Q T V S K N R A D L T E M V K K I T   | 1801 |
| 5795 AGC ATG AAC AGG GGA GAC TTC AAG CAG ATT ACT TTG GCA ACA GGG GCA GGC AAA ACC ACA     | 5854 |
| 1802 S M N R G D F K Q I T L A T G A G K T T   | 1821 |
| 5855 GAA CTC CCA AAA GCA GTT ATA GAG GAG ATA GGA AGA CAC AAG AGA GTA TTA GTT CTT ATA     | 5914 |
| 1822 E L P K A V I E E I G R H K R V L V L I   | 1841 |
| 5915 CCA TTA AGG GCA GCG GCA GAG TCA GTC TAC CAG TAT ATG AGA TTG AAA CAC CCA AGC ATC     | 5974 |
| 1842 P L R A A A E S V Y Q Y M R L K H P S I   | 1861 |
| 5975 TCT TTT AAC CTA AGG ATA GGG GAC ATG AAA GAG GGG GAC ATG GCA ACC GGG ATA ACC TAT     | 6034 |
| 1862 S F N L R I G D M K E G D M A T G I T Y   | 1881 |
| 6035 GCA TCA TAC GGG TAC TTC TGC CAA ATG CCT CAA CCA AAG CTC AGA GCT GCT ATG GTA GAA     | 6094 |
| 1882 A S Y G Y F C Q M P Q P K L R A A M V E   | 1901 |
| 6095 TAC TCA TAC ATA TTC TTA GAT GAA TAC CAT TGT GCC ACT CCT GAA CAA CAA CTG GCA ATT ATC | 6154 |
| 1902 Y S Y I F L D E Y H C A T P E Q L A I I   | 1921 |
| 6155 GGG AAG ATC CAC AGA TTT TCA GAG AGT ATA AGG GTT GTC GCC ATG ACT GCC ACG CCA GCA     | 6214 |
| 1922 G K I H R F S E S I R V V A M T A T P A   | 1941 |
| 6215 GGG TCG GTG ACC ACA ACA GGT CAA AAG CAC CCA ATA GAG GAA TTC ATA GCC CCC GAG GTA     | 6274 |
| 1942 G S V T T T G Q K H P I E E F I A P E V   | 1961 |
| 6275 ATG AAA GGG GAG GAT CTT GGT AGT CAG TTC CTT GAT ATA GCA GGG TTA AAA ATA CCA GTG     | 6334 |
| 1962 M K G E D L G S Q F L D I A G L K I P V   | 1981 |
| 6335 GAT GAG ATG AAA GGC AAT ATG TTG GTT TTT GTC CCA ACN AGA AAC ATG GCA GTC GAG GTA     | 6394 |
| 1982 D E M K G N M L V F V P T R N M A V E V   | 2001 |
| 6395 GCA AAG AAG CTA AAA GCT AAG GGC TAT AAC TCT GGA TAC TAT TAC AGT GGA GAG GAT CCA     | 6454 |
| 2002 A K K L K A K G Y N S G Y Y Y S G E D P   | 2021 |
| 6455 GCC AAT CTG AGA GTT GTG ACA TCA CAA TCC CCC TAT GTC ATC GTG GCT ACA AAT GCT ATT     | 6514 |
| 2022 A N L R V V T S Q S P Y V I V A T N A I   | 2041 |
| 6515 GAA TCA GGA GTG ACA CTA CCA GAT TTG GAC ACG GTT ATA GAC ACG GGG TTG AAA TGT GAA     | 6574 |
| 2042 E S G V T L P D L D T V I D T G L K C E   | 2061 |
| 6575 AAG AGG GTG AGG GTA TCA TCA AAG ATA CCC TTC ATC GTC ACA GCA GGC CTT AAG AGG ATG GCC | 6634 |
| 2062 K R V R V S S K I P F I V T G L K R M A   | 2081 |
| 6635 GTG ACT GTG GGT GAG CAG GCG CAG CGT AGG GGC AGA GTA GGT AGA GTG AAA CCC GGG AGG     | 6694 |
| 2082 V T V G E Q A Q R R G R V G R V K P G R   | 2101 |
| 6695 TAT TAT AGG AGC CAG GAA ACA GCA ACA GGG TCA AAG GAC TAC CAC TAT GAC CTC TTG CAG     | 6754 |
| 2102 Y Y R S Q E T A T G S K D Y H Y D L L Q   | 2121 |
| 6755 GCA CAA AGA TAC GGG ATT GAG GAT GGA ATC AAC GTG AGC AAA TCC TTT AGG GAG ATG AAT     | 6814 |
| 2122 A Q R Y G I E D G I N V T K S F R E M N   | 2141 |
| 6815 TAC GAT TGG AGC CTA TAC GAG GAG GAC AGC CTA CTA ATA ACC CAG CTG GAA ATA CTA AAT     | 6874 |
| 2142 Y D W S L Y E E D S L L I T Q L E I L N   | 2161 |
| 6875 AAT CTA CTC ATC TCA GAA GAC TTG CCA GCC GCT GTT AAG AAC ATA ATG GCC AGG ACT GAT     | 6934 |
| 2162 N L L I S E D L P A A V K N I M A R T D   | 2181 |
| 6935 CAC CCA GAG CCA ATC CAA CTT GCA TAC AAC AGC TAT GAA GTC CAG GTC CCG GTC CTG TTC     | 6994 |
| 2182 H P E P I Q L A Y N S Y E V Q V P V L F   | 2201 |

FIGURE 12-4

| BVDV NADL clns- (inf. clone)   | Genes | 34/67 | 4/21/99 | 5:45:24 PM | Page 5 |
|--|-------|-------|---------|------------|--------|
| 6995 CCA AAA ATA AGG AAT GGA GAA GTC ACA GAC ACC TAC GAA AAT TAC TCG TTT CTA AAT GCC |       | 7054  |         |            |        |
| 2202 P K I R N G E V T D T Y E N Y S F L N A   |       | 2221  |         |            |        |
| 7055 AGA AAG TTA CGG GAG GAT GTG CCC GTG TAT ATC TAC GCT ACT GAA GAT GAG GAT CTG GCA |       | 7114  |         |            |        |
| 2222 R K L G E D V P V Y I Y A T E D E D L A   |       | 2241  |         |            |        |
| 7115 GTT GAC CTC TTA CGG CTA GAC TGG CCT GAT CCT GGG AAC CAG CAG GTA GTG GAG ACT GGT |       | 7174  |         |            |        |
| 2242 V D L L G L D W P D P G N Q Q V V E T G   |       | 2261  |         |            |        |
| 7175 AAA GCA CTG AAG CAA GTG ACC GGG TTG TCC TCG GCT GAA AAT GCC CTA CTA GTG GCT TTA |       | 7234  |         |            |        |
| 2262 K A L K Q V T G L S S A E N A L L V A L   |       | 2281  |         |            |        |
| 7235 TTT GGG TAT GTG GGT TAC CAG GCT CTC TCA AAG AGG CAT GTC CCA ATG ATA ACA GAC ATA |       | 7294  |         |            |        |
| 2282 F G Y V G Y Q A L S K R H V P M I T D I   |       | 2301  |         |            |        |
| 7295 TAT ACC ATC GAG GAC CAG AGA CTA GAA GAC ACC ACC CAC CTC CAG TAT GCA CCC AAC GCC |       | 7354  |         |            |        |
| 2302 Y T I E D Q R L E D T T H L Q Y A P N A   |       | 2321  |         |            |        |
| 7355 ATA AAA ACC GAT GGG ACA GAG ACT GAA CTG AAA GAA CTG GCG TCG GGT GAC GTG GAA AAA |       | 7414  |         |            |        |
| 2322 I K T D G T E T E L K E L A S G D V E K   |       | 2341  |         |            |        |
| 7415 ATC ATG CGA GCC ATT TCA GAT TAT GCA GCT GGG GGA CTG GAG TTT GTT AAA TCC CAA GCA |       | 7474  |         |            |        |
| 2342 I M G A I S D Y A A G G L E F V K S Q A   |       | 2361  |         |            |        |
| 7475 GAA AAG ATA AAA ACA GCT CCT TTG TTT AAA GAA AAC GCA GAA GCC GCA AAA GGG TAT GTC |       | 7534  |         |            |        |
| 2362 E K I K T A P L F K E N A E A A K G Y V   |       | 2381  |         |            |        |
| 7535 CAA AAA TTC ATT GAC TCA TTA ATT GAA ATT AAA GAA GAA ATA ATC AGA TAT GGT TTG TGG |       | 7594  |         |            |        |
| 2382 Q K F I D S L I E N K E E I I R Y G L W   |       | 2401  |         |            |        |
| 7595 GGA ACA CAC ACA GCA CTA TAC AAA AGC ATA GCT GCA AGA CTG GGG CAT GAA ACA GCG TTT |       | 7654  |         |            |        |
| 2402 G T H T A L Y K S I A A R L G H E T A F   |       | 2421  |         |            |        |
| 7655 GCC ACA CTA GTG TTA AAG TGG CTA GCT TTT GGA GGG GAA TCA GTG TCA GAC CAC GTC AAG |       | 7714  |         |            |        |
| 2422 A T L V L K W L A F G G E S V S D H V K   |       | 2441  |         |            |        |
| 7715 CAG GCG GCA GTT GAT TTA GTG GTC TAT TAT GTG ATG AAT AAG CCT TCC TTC CCA GGT GAC |       | 7774  |         |            |        |
| 2442 Q A A V D L V V Y Y V M N K P S F P G D   |       | 2461  |         |            |        |
| 7775 TCC GAG ACA CAG CAA GAA GGG AGG CGA TTC GTC GCA AGC CTG TTC ATC TCC GCA CTG GCA |       | 7834  |         |            |        |
| 2462 S E T Q Q E G R R F V A S L F I S A L A   |       | 2481  |         |            |        |
| 7835 ACC TAC ACA TAC AAA ACT TGG AAT TAC CAC AAT CTC TCT AAA GTG GTG GAA CCA GCC CTG |       | 7894  |         |            |        |
| 2482 T Y T Y K T W N Y H N L S K V V E P A L   |       | 2501  |         |            |        |
| 7895 GCT TAC CTC CCC TAT GCT ACC AGC GCA TTA AAA ATG TTC ACC CCA ACG CGG CTG GAG AGC |       | 7954  |         |            |        |
| 2502 A Y L P Y A T S A L K M F T P T R L E S   |       | 2521  |         |            |        |
| 7955 GTG GTG ATA CTG AGC ACC AGC ATA TAT AAA ACA TAC CTC TCT ATA AGG AAG GGG AAG AGT |       | 8014  |         |            |        |
| 2522 V V I L S T T I Y K T Y L S I R K G K S   |       | 2541  |         |            |        |
| 8015 GAT GGA TTG CTG GGT ACG GGG ATA AGT GCA GCC ATG GAA ATC CTG TCA CAA AAC CCA GTA |       | 8074  |         |            |        |
| 2542 D G L L G T G I S A A M E I L S Q N P V   |       | 2561  |         |            |        |
| 8075 TCG GTA GGT ATA TCT GTG ATG TTG GGG GTA GGG GCA ATC GCT GCG CAC AAC GCT ATT GAG |       | 8134  |         |            |        |
| 2562 S V G I S V M L G V G A I A A H N A I E   |       | 2581  |         |            |        |
| 8135 TCC ACT GAA CAG AAA AGG ACC CTA CTT ATG AAG GTG TTT GTA AAG AAC TTC TTG GAT CAG |       | 8194  |         |            |        |
| 2582 S S E Q K R T L L M K V F V K N F L D Q   |       | 2601  |         |            |        |
| 8195 GCT GCA ACA GAT GAG CTG GTC AAA GAA AAC CCA GAA AAA ATT ATA ATG GCC TTA TTT GAA |       | 8254  |         |            |        |
| 2602 A A T D E L V K E N P E K I I M A L F E   |       | 2621  |         |            |        |
| 8255 GCA GTC CAG ACA ATT CGT AAC CCC CTG AGA CTA ATA TAC CAC CTG TAT GGG GTT TAC TAC |       | 8314  |         |            |        |
| 2622 A V Q T I G N P L R L I Y H L Y G V Y Y   |       | 2641  |         |            |        |
| 8315 AAA GGT TGG GAG GCC AAG GAA CTA TCT GAG AGG ACA GCA GGC AGA AAC TTA TTC ACA TTG |       | 8374  |         |            |        |
| 2642 K G W E A K E L S E R T A G R N L F T L   |       | 2661  |         |            |        |
| 8375 ATA ATG TTT GAA GCC TTC GAG TTA TTA GGG ATG GAC TCA CAA GGG AAA ATA AGG AAC CTG |       | 8434  |         |            |        |
| 2662 I M F E A F E L L L G M D S O G K I R N L                                       |       | 2681  |         |            |        |
| 8435 TCC GGA AAT TAC ATT TTG GAT TTG ATA TAC GGC CTA CAC AAG CAA ATC AAC AGA GGG CTG |       | 8494  |         |            |        |
| 2682 S G N Y I L D L I Y G L H K Q I N R G L   |       | 2701  |         |            |        |
| 8495 AAG AAA ATG GTC CTG GGG TGG GCC CCT GCA CCC TTT AGT TGT GAC TGG ACC CCT AGT GAC |       | 8554  |         |            |        |
| 2702 K K M V L G W A P A P F S C D W T P S D   |       | 2721  |         |            |        |
| 8555 GAG AGG ATC AGA TTG CCA ACA GAC AAC TAT TTG AGG GTA GAA ACC AGG TGC CCA TGT GGC |       | 8614  |         |            |        |
| 2722 E R I R L P T D N Y L R V E T R C P C G   |       | 2741  |         |            |        |
| 8615 TAT GAG ATG AAA GCT TTC AAA AAT GTA GGT GGC AAA CTT ACC AAA GTG GAG GAG AGC GGG |       | 8674  |         |            |        |
| 2742 Y E M K A F K N V G G K L T K V E E S G   |       | 2761  |         |            |        |
| 8675 CCT TTC CTA TGT AGA AAC AGA CCT GGT AGG GGA CCA GTC AAC TAC AGA GTC ACC AAG TAT |       | 8734  |         |            |        |
| 2762 P F L C R N R P G R G P V N Y R V T K Y   |       | 2781  |         |            |        |

FIGURE 12-5

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BVDV NADL clns- (inf. clone) Genes

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|   |               |
|---|---------------|
| 8735 TAC GAT GAC AAC CTC AGA GAG ATA AAA CCA GTC GCA AAG TTG GAA GGA CAG GTA GAG CAC<br>2782 Y D D N L R E I K P V A K L E G Q V E H  | 8794<br>2801  |
| 8795 TAC TAC AAA GGG GTC ACA GCA AAA ATT GAC TAC AGT AAA GGA AAA ATG CTC TTG GCC ACT<br>2802 Y Y K G V T A K I D Y S K G K M L L A T  | 8854<br>2821  |
| 8855 GAC AAG TGG GAG GTG GAA CAT GGT GTC ATA ACC AGG TTA GCT AAG AGA TAT ACT GGG GTC<br>2822 D K W E V E H G V I T R L A K R Y T G V  | 8914<br>2841  |
| 8915 GGG TTC AAT GGT GCA TAC TTA GGT GAC GAG CCC AAT CAC CGT CCT CTA GTG GAG AGG GAC<br>2842 G F N G A Y L G D E P N H R A L V E R D  | 8974<br>2861  |
| 8975 TGT GCA ACT ATA ACC AAA AAC ACA GTC CAG TTT CTA AAA ATG AAG AAG GGG TGT GCG TTC<br>2862 C A T I T K N T V Q F L K M K K G C A F  | 9034<br>2881  |
| 9035 ACC TAT GAC CTG ACC ATC TCC AAT CTG ACC AGG CTC ATC GAA CTA GTA CAC AGG AAC AAT<br>2882 T Y D L T I S N L T R L I E L V H R N N  | 9094<br>2901  |
| 9095 CTT GAA GAG AAG GAA ATA CCC ACC GCT ACG GTC ACC ACA TGG CTA GCT TAC ACC TTC GTG<br>2902 L E E K E I P T A T V T T W L A Y T F V  | 9154<br>2921  |
| 9155 AAT GAA GAC GTC GGG ACT ATA AAA CCA GTC CTA GGA GAG AGA GTC ATC CCC GAC CCT GTC<br>2922 N E D V G T I K P V L G E R V I P D P V  | 9214<br>2941  |
| 9215 GTT GAT ATC AAT TTA CAA CCA GAG GTG CAA GTG GAC ACG TCA GAG GTT GGG ATC ACA ATA<br>2942 V D I N L Q P E V Q V D T S E V G I T I  | 9274<br>2961  |
| 9275 ATT GGA AGG GAA ACC CTG ATG ACA ACG GGA GTG ACA CCT GTC TTG GAA AAA GTC GAG CCT<br>2962 I G R E T L M T T G V T P V L E K V E P  | 9334<br>2981  |
| 9335 GAC GCC AGC GAC AAC CAA AAC TCG GTG AAG ATC GGG TTG GAT GAG GGT AAT TAC CCA GGG<br>2982 D A S D N Q N S V K I G L D E G N Y P G  | 9394<br>3001  |
| 9395 CCT GGA ATA CAG ACA CAT ACA CTA ACA GAA GAA ATA CAC AAC AGG GAT GCG AGG CCC TTC<br>3002 P G I Q T H T L T E E I H N R D A R P F  | 9454<br>3021  |
| 9455 ATC ATG ATC CTG CGC TCA AGG AAT TCC ATA TCA AAT AGG GCA AAG ACT GCT AGA AAT ATA<br>3022 I M I L G S R N S I S N R A K T A R N I  | 9514<br>3041  |
| 9515 AAT CTG TAC ACA CGG AAT GAC CCC AGG GAA ATA CGA GAC TTG ATG GCT GCA GGG CGC ATG<br>3042 N L Y T G N D P R E I R D L M A A G R M  | 9574<br>3061  |
| 9575 TTA GTA GCA CGT AGG GAT GTC GAC CCT GAG CTG TCT GAA ATG GTC GAT TTC AAG GGG<br>3062 L V V A L R D V D P E L S E M V D F K G      | 9634<br>3081  |
| 9635 ACT TTT TTA GAT AGG GAG GCC CTG GAG GCT CTA AGT CTC GGG CAA CCT AAA CCG AAG CAG<br>3082 T F L D R E A L E A L S L G Q P K P K Q  | 9694<br>3101  |
| 9695 GTT ACC AAG GAA GCT GTT AGG AAT TTG ATA GAA CAG AAA AAA GAT GTG GAG ATC CCT AAC<br>3102 V T K E A V P N L I E Q K K P V E I P N  | 9754<br>3121  |
| 9755 TGG TTT GCA TCA GAT GAC CCA GTC TTT CTG GAA GTG GCC TTA AAA AAT GAT AAG TAC TAC<br>3122 W F A S D D P V F L E V A L K N D K Y Y  | 9814<br>3141  |
| 9815 TTA GTA GGA GAT GTT GGA GAG CTA AAA GAT CAA GCT AAA GCA CTT GGG GCC ACG GAT CAG<br>3142 L V G D V G E L K D Q A K A L G A T D Q  | 9874<br>3161  |
| 9875 ACA AGA ATT ATA AAG GAG GTA GGC TCA AGG ACG TAT GCC ATG AAG CTA TCT AGC TGG TTC<br>3162 T R I I K E V G S R T Y A M K L S S W F  | 9934<br>3181  |
| 9935 CTC AAG GCA TCA AAC AAA CAG ATG AGT TTA ACT CCA CTG TTT GAG GAA TTG TTG CTA CGG<br>3182 L K A S N K Q M S L T P L F E E L L L R  | 9994<br>3201  |
| 9995 TGC CCA CCT GCA ACT AAG AGC AAT AAG GGG CAC ATG GCA TCA GCT TAC CAA TTG GCA CAG<br>3202 C P P A T K S N K G H M A S A Y Q L A Q  | 10054<br>3221 |
| 10055 GGT AAC TGG GAG CCC CTC GGT TGC GGG GTG CAC CTA CGT ACA ATA CCA GCC AGA AGG GTG<br>3222 G N W E P L G C G V H L G T I P A R R V | 10114<br>3241 |
| 10115 AAG ATA CAC CCA TAT GAA GCT TAC CTG AAG TTG AAA GAT TTC ATA GAA GAA GAA GAG AAG<br>3242 K I H P Y E A Y L K L K D F I E E E E K | 10174<br>3261 |
| 10175 AAA CCT AGG GTT AAG GAT ACA GTC ATA AGA GAG CAC AAC AAA TGG ATA CTT AAA AAA ATA<br>3262 K P R V K D T V I R E H N K W I L K K I | 10234<br>3281 |
| 10235 AGG TTT CAA GGA AAC CTC AAC ACC AAG AAA ATG CTC AAC CGG GGG AAA CTA TCT GAA CAG<br>3282 R F Q G N L N T K K M L N P G K L S E Q | 10294<br>3301 |
| 10295 TTG GAC AGG GAG GGG CGC AAG AGG AAC ATC TAC AAC CAC CAG ATT GGT ACT ATA ATG TCA<br>3302 L D R E G R K R N I Y N H Q I G T I M S | 10354<br>3321 |
| 10355 AGT GCA GGC ATA AGG CTG GAG AAA TTG CCA ATA GTG AGG GCC CAA ACC GAC ACC AAA ACC<br>3322 S A G I R L E K L P I V R A Q T D T K T | 10414<br>3341 |
| 10415 TTT CAT GAG GCA ATA AGA GAT AAG ATA GAC AAG AGT GAA AAC CGG CAA AAT CCA GAA TTG<br>3342 F H E A I R D K I D K S E N R Q N P E L | 10474<br>3361 |

FIGURE 12-6

| BVDV NADL cins- (inf. clone)  | Genes  | 36/67      | 4/21/99 | 5:45:24 PM | Page 7 |
|---|--|------------|---------|------------|--------|
| 10475 CAC AAC AAA TTG TTG GAG ATT TTC CAC ACG ATA GCC CAA CCC ACC CTG AAA CAC ACC TAC       | 3362 H N K L L E I F H T I A Q P T L K H T Y   | 10534 3381 |         |            |        |
| 10535 GGT GAG GTG ACG TGG GAG CAA CTT GAG GCG GGG ATA AAT AGA AAG GGG GCA GCA GGC TTC       | 3382 G E V T W E Q L E A G I N R K G A A G F   | 10594 3401 |         |            |        |
| 10595 CTG GAG AAG AAC ATC GGA GAA GTA TTG GAT TCA GAA AAG CAC CTG GTA GAA CAA TTG           | 3402 L E K K N I G E V L D S E K H L V E Q L   | 10654 3421 |         |            |        |
| 10655 GTC AGG GAT CTG AAG GCC GGG AGA AAG ATA AAA TAT TAT GAA ACT GCA ATA CCA AAA AAT       | 3422 V R D L K A G R K I K Y Y E T A I P K N   | 10714 3441 |         |            |        |
| 10715 GAG AAG AGA GAT GTC AGT GAT GAC TGG CAG GCA GGG GAC CTG GTG GTT GAG AAG AGG CCA       | 3442 E K R D V S D D W Q A G D L V V E K R P   | 10774 3461 |         |            |        |
| 10775 AGA GTT ATC CAA TAC CCT GAA GCC AAG ACA AGG CTA GCC ATC ACT AAG GTC ATG TAT AAC       | 3462 R V I Q Y P E A K T R L A I T K V M Y N   | 10834 3481 |         |            |        |
| 10835 TGG GTG AAA CAG CAG CCC GTT GTG ATT CCA GGA TAT GAA GGA AAG ACC CCC TTG TTC AAC       | 3482 W V K Q Q P V V I P G Y E G K T P L F N   | 10894 3501 |         |            |        |
| 10895 ATC TTT GAT AAA GTG AGA AAG GAA TGG GAC TCG TTC AAT GAG CCA GTG GCC GTA AGT TTT       | 3502 I F D K V R K E W D S F N E P V A V S F   | 10954 3521 |         |            |        |
| 10955 GAC ACC AAA GCC TGG GAC ACT CAA GTG ACT AGT AAG GAT CTG CAA CTT ATT GGA GAA ATC       | 3522 D T K A W D T Q V T S K D L Q L I G E I   | 11014 3541 |         |            |        |
| 11015 CAG AAA TAT TAC TAT AAG AAG GAG TGG CAC AAG TTC ATT GAC ACC ATC ACC GAC CAC ATG       | 3542 Q K Y Y Y K K E W H K F I D T I T D H M   | 11074 3561 |         |            |        |
| 11075 ACA GAA GTA CCA GTT ATA ACA GCA GAT GGT GAA GTA TAT ATA AGA AAT GGG CAG AGA GGG       | 3562 T E V P V I T A D G E V Y I R N G Q R G   | 11134 3581 |         |            |        |
| 11135 AGC GGC CAG CCA GAC ACA AGT GCT GGC AAC AGC ATG TTA AAT GTC CTG ACA ATG ATG TAC       | 3582 S G Q P D T S A G N S M L N V L T M M Y   | 11194 3601 |         |            |        |
| 11195 GGC TTC TGC GAA AGC ACA GGG GTA CCG TAC AAG AGT TTC AAC AGG GTG GCA AGG ATC CAC       | 3602 G F C E S T G V P Y K S F N R V A R I H   | 11254 3621 |         |            |        |
| 11255 GTC TGT GGG GAT GAT GGC TTC TTA ATA ACT GAA AAA GGG TTA GGG CTG AAA TTT GCT AAC       | 3622 V C G D D G F L I T E K G L G L K F A N   | 11314 3641 |         |            |        |
| 11315 AAA GGG ATG CAG ATT CTT CAT GAA GCA GGC AAA CCT CAG AAG ATA ACG GAA GGG GAA AAG       | 3642 K G M Q I L H E A G K P Q K I T E G E K   | 11374 3661 |         |            |        |
| 11375 ATG AAA GTT GCC TAT AGA TTT GAG GAT ATA GAG TTC TGT TCT CAT ACC CCA GTC CCT GTT       | 3662 M K V A Y R F E D I E F C S H T P V P V   | 11434 3681 |         |            |        |
| 11435 AGG TGG TCC GAC AAC ACC AGT CAC ATG GCC GGG AGA GAC ACC GCT GTG ATA CTA TCA           | 3682 R W S D N T S S H M A G R D T A V I L S   | 11494 3701 |         |            |        |
| 11495 AAG ATG GCA ACA AGA TTG GAT TCA AGT GGA GAG AGG GGT ACC ACA GCA TAT GAA AAA GCG       | 3702 K M A T R L D S S G E R G T T A Y E K A   | 11554 3721 |         |            |        |
| 11555 GTA GCC TTC AGT TTC TTG CTG ATG TAT TCC TGG AAC CCG CTT GTT AGG AGG ATT TGC CTG       | 3722 V A F S F L L M Y S W N P L V R R I C L   | 11614 3741 |         |            |        |
| 11615 TTG GTC CTT TCG CAA CAG CCA GAG ACA GAC CCA TCA AAA CAT GCC ACT TAT TAT TAC AAA       | 3742 L V L S Q Q P E T D P S K H A T Y Y K     | 11674 3761 |         |            |        |
| 11675 GGT GAT CCA ATA GGG GGC TAT AAA GAT GTA ATA GGT CGG AAT CTA AGT GAA CTG AAG AGA       | 3762 G D P I G A Y K D V I G R N L S E L K R   | 11734 3781 |         |            |        |
| 11735 ACA GGC TTT GAG AAA TTG GCA AAT CTA AAC CTA AGC CTG TCC ACG TTG GGG ATC TGG ACT       | 3782 T G F E K L A N L N L S L S T L G I W T   | 11794 3801 |         |            |        |
| 11795 AAG CAC ACA AGC AAA AGA ATA ATT CAG GAC TGT GTT GCC ATT GGG AAA GAA GAG GGC AAC       | 3802 K H T S K R I I Q D C V A I G K E E G N   | 11854 3821 |         |            |        |
| 11855 TGG CTA GTT AAC GCC GAC AGG CTG ATA TCC AGC AAA ACT GGC CAC TTA TAC ATA CCT GAT       | 3822 W L V N A D R L I S S S K T G H L Y I P D | 11914 3841 |         |            |        |
| 11915 AAA GGC TTT ACA TTA CAA GGA AAG CAT TAT GAG CAA CTG CAG CTA AGA ACA GAG ACA AAC       | 3842 K G F T L Q G K H Y E Q L Q L R T E T N   | 11974 3861 |         |            |        |
| 11975 CCG GTC ATG GGG GTT GGG ACT GAG AGA TAC AAG TTA GGT CCC ATA GTC AAT CTG CTG CTG       | 3862 P V M G V G T E R Y K L G P I V N L L L   | 12034 3881 |         |            |        |
| 12035 AGA AGG TTG AAA ATT CTG CTC ATG ACG GCC GTC GGC GTC AGC ACC TGA gacaaaatgtatata       | 3882 R R L K I L L M T A V G V S S             | 12098 3897 |         |            |        |
| 12099 tgtaaaatataatccatgtacatagtgtatataatagttggaccgcgtccacctaagaagacgacacgcactttagctgtatgag |  | 12178      |         |            |        |
| 12179 cgcacagctaaacagttagtcaagattatctacctaagataaacactacatataatgcacacagcacttagctgtatgag      |  | 12258      |         |            |        |
| 12259 gatacgccccgacgtctatagttggacttagggaaagacctctaaacagcccc                                 |  | 12308      |         |            |        |

FIGURE 12-7

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GTATaatcactccctgtgaggaactacgtttcacgcagaaggcgtagccatggcgtagttagtgagtgtcggtgcagcccccag  
gccccccctcccgggagagccatagtggctgcggaaaccggggagtgacaccggaaattgccaggacgaccggggcccttcgtggata  
aacccgctcaatgcctggagattggcgigcccccaagactgtagccgagtagtggtgggtcgcgaaaggccctgggtactgc  
ctgatagggtgcgtgcgagtgcggggaggctcgttagaccgtgcaccATG

**FIGURE 13**

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GTaatcactccccgtgaggaactactgtttcacgcagaaaagcgtagccatggcgtagtatgagtgtcgccagcccccaggac  
ccccccctcccgggagagccatagtggctgcggaccggtgagtaaccggaaattgccaggacgaccgggtccttcttggataaac  
ccgcitcaatccctggagattggcgtagcccccgcaagactgctagccgagtagtgtgggtcgccaaaggccgtgtggtaatgcctg  
atagggtgcgtcgagtgccccggaggctcgtagaccgtgcaccATG

**FIGURE 14**

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GTA  
TAcactccaccatgaatcactcccctgtgaggaactactgtcttcacgcaga  
aaagcgtagccatggcgtagttagttagtgatgtcg  
tgcaggcctccaggaccccccccccggagagccatagggctgcgg  
aaccggtagtacaccggaattgccaggacgcaccgg  
tcccttcggataaaacccgtcaatgcggagattggcg  
tgcgcggcccccgaagactgttagccgagtagt  
gttgggtcgcgaaaggc  
tttgttactgcctgatagggtgcgagtgccccggagg  
tgcgttagaccgtgcaccATG

**FIGURE 15**

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GTATCAGAAGTGCAGATGCTGAacactccaccatgaatcactccctgtgaggaactactgtttcacgcagaaa  
gcgtctagccatggcgtagttagtgcgtgcagcctccaggcccccccggagagccatagtggctcgccgaaccggtg  
agtacaccggaaatgccaggacgaccgggccttcgtggataaaaccgcgtcaatgcctggagatttggcgtgcccccaagactg  
ctagccgagtagtgtgggtcgcgaaaggcctgtggtaactgcctgtatagggtgcctgcgagtgccccggaggtctcgttagaccgtg  
caccATG

**FIGURE 16**

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GTATgccagccccctgatgggggcgacactccaccatgaatcactccctgtgaggaactactgtttcacgcagaaggcgcttag  
ccatggcgtagttagtgagtgtcgltgcagcccccaggaccctccggagagccatagtggctlgcggaaaccggtagtacacc  
ggaattgccaggacgaccgggtcccttcggataaaccgcctaattgcctggagattggcgtgcccccaagactgttagccgat  
gtatgttgggtcgcgaaaggccctgtggtaactgcctgtatagggtgtcgagtgccccgggaggctcgtagaccgtgcaccAT  
G

**FIGURE 17**

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GTATTGCAGTTgccagccccctgatggggcgacactccaccatgaatcactcccccgtgaggaactactgtctcacgc  
agaaagcgctctagccatggcgtagtatgagtgctgcgcgcctccaggacccccccctccggagagccatagtggctcgccggaaac  
cggtgagtacccggaattccaggacgaccgggtcccttctggataaaccgcctcaatgcctggagattggcggtgcggccggaaac  
gactgcctagccgagatgtgtgggtcgcgaaaggccctgtggtaactgcctgatagggcgctgcgagtgccccgggaggctcgtaga  
ccgtgcaccATG

**FIGURE 18**

GTATTGCAGTTgccagccccctgatggggcgacactccaccatgaatcactccctgtgaggaactactgtttcacgc  
agaaaaagcgtctagccatggcgtagttagtgatgtcggtcgacgcctccaggaccccccccctccggagagccatagggctgcccgaac  
cggtaggtacaccggaaatggcaggacggccggcccttctggataaaccggctcaatgcctggagattggcggtgcccggagggtctcgtaga  
gactgttagccgagtagtgtgggtcgcaagggctgtggtaatgggtctgcgagtgcccggagggtctcgtaga  
ccgtgcaccATGGAGTTGATCACAAATGAACCTTATACAAAACATACAAACAAAAAC  
CCGTCGGGGTGGAGGAACCTGTTATGATCAGGCAGGTGATCCCTTATTGGT  
GAAAGGGGAGCAGTCCACCCTCAATCGACGCTAAAGCTCCCACACAAGAGAG  
GGGAACGCGATGTTCCAACCAACTGGCATCCTTACCAAAAAAGAGGTGACTGC  
AGGTGGTAATAGCAGAGGACCTGTGAGCGGGATCTACCTGAAGGCCAGGGC  
CACTATTACCGAGACTATAAAGGTCCCGTCTATCACAGGGCCCCCTGGAGC  
TCATTGAGGAGGGATCCATGTGAAACAGACTAACCGATAAGGGAGAGTAAC  
GGAAGTGACGAAAGCTGTACCCACATTATGTGTGATAGATGGATGTATAATA  
ATAAAAAAAGTGCACAGAGAAGTACCAAAAGGGTGTCAAGGTGGTCCATAATAG  
GCTTGAETGCCCTCATGGTCAAACTTGTCTAGACACGAAAGAAGAGGGAG  
CAACAAAAAAAGAAAACACAGAAACCCGACAGACTAGAAAGGGGAAAATGAA  
AATAGTGCCAAAGAAATCTGAAAAAAGACAGCAAAACTAACCTCCGGATGCTA  
CAATAGTGGTGGAAAGGAGTCAAATACCAGGTGAGGAAGAAGGGAAAACCAA  
GAGTAAAAAACACTCAGGACGGCTTGTACCCATAACAAAAACACTCAGGAAT  
CACGCAAGAAACTGGAAAAGCATTGTTGGCGTGGCAATAATAGCTATAGTT  
TTGTTCAAGTTACAATGGGAGAAAACATAACACAGTGGAACCTACAAGATAAT  
GGGACGGAAGGGATAACACGGCAATGTTCAAAGGGGTGTGAATAGAAGTT  
TACATGGAAATCTGCCAGAGAAAATCTGTACTGGTGTCCCTCCATCTAGCCA  
CCGATATAGAACTAAAAACAATTCATGGTATGATGGATGCAAGTGAGAAGACC  
AACTACACGTGTTGCAGACTTCAACGCCATGAGTGGAAACAAGCATGGTGGTG  
CAACTGGTACAATATTGAACCTGGATTCTAGTCATGAATAGAACCCAGCCAA  
TCTCACTGAGGGACAACCACCAAGGGAGTGCAGTCATTGTAGGTATGATA  
GGGCTAGTGAACACGTGGTAACACAAGCTAGAGATAGCCCCACACCCCTA  
ACAGGTTGCAAGAAAGGAAAGAACCTTCTCCTTGCAGGCATATTGATGCGGGG  
CCCTGCAACTTGAATAGCTGCAAGTGATGTATTATTCAAAGAACATGAACG  
CATTAGTATGTTCCAGGATACTACTCTTACCTTGTGACGGTTGACCAACTCC  
TTAGAAGGTGCCAGACAAGGAACCGCTAAACTGACAACCTGGTTAGGCAAGCA  
GCTCGGGATACTAGGAAAAAGTTGAAAACAAGAGTAAGACGTGGTTGGAG  
CATACGCTGTTCCCTACTGTGATGTCATGCAAAATTGGCTACATATGGT  
ATACAAAAAAATTGACACCAATGCAAGAGGACGGCAAGATATTACATGAGATGGG  
CTGGGAAATTGACACCAATGCAAGAGGACGGCAAGATATTACATGAGATGGG  
GGTCACTTGTGGAGGTACTACTACTTTCTTAGTGGTGTGCTGACTCGCA  
CCGGAAACAGCTAGTGAATGTACCTAACCTACATTTCATCCCACAGTGGAGCTG  
ACGTTGATGTAATGGATTGTGATAAGACCCAGTTGAACCTCACAGTGGAGCTG  
ACAACAGCTGAAGTAATACCAAGGGTGGCTGGAAATCTAGGCAAATATGTATG  
TATAAGACCAAAATTGGTGGCCTTATGAGACAACCTGATGTGGCATTTGAAGA  
GGTAGGGCAGGTGGTGAAGTTAGTGGTGGGCACTCAGAGATTTAACACGCA  
TTGGAAACGCTGCAACAACTACTGCTTTTGTAGTATGCCTTGTAAAGATAGTCAG  
GGGCCAGATGGTACAGGGCATTCTGTGGCTACTATTGATAACAGGGTACAAG  
GGCACTGGATTGCAACACCTGAATTCTCGTATGCCATAGCAAAGGACGAAAGA  
ATTGGTCAACTGGGGCTGAAGGCCTTACCAACACTGGAAAGGAATACTCACC  
TGGAAATGAAGCTGGAAGACACAAATGGTCAATTGCTTGGTGCAGAGATGGGAAGT  
TAATGTACCTCCAAAGATGCACTGAGAGAAACCAGGTATCTCGCAATCTTGCATA  
CAAGAGCCTTGCCGACCAGTGTGGTATTCAAAAACCTTTGATGGCGAAAG

CAAGAGGATGTAGTCGAAATGAACGACAACITTGAAATTGGACTCTGCCCATGT  
GATGCCAAACCCATAGTAAGAGGGAAAGTCAATAACACGCTGCTGAACGGACC  
GGCCTTCAGATGGTATGCCCATAGGATGGACAGGGACTGTAAGCTGTACGT  
CATTCAATATGGACACCTTAGCCACAACCTGTTACGGACATATAGAAGGTCTA  
AACCATTCCCTCATAGGCAAGGCTGTATCACCCAAAAGAATCTGGGGAGGAT  
CTCCATAACTGCATCCTGGAGGAAATTGGACTTGTGCTGGAGACCAACTA  
CTATACAAAGGGGCTCTATTGAATCTTCAAGTGGTGTGGCTATCAATTAAA  
GAGAGTGAGGGACTACCACACTACCCATTGGCAAGTGTAAATTGGAGAACGA  
GACTGGTTACAGGCTAGTAGACAGTACCTCTTCAATAGAGAAGGTGTGGCCA  
TAGTACACAAAGGGACATTAAGTCAAGATAGGAAAACAACGTACAGGTC  
ATAGCTATGGATACCAAACCTGGACCTATGCCCTGCAGACCATATGAAATCATA  
TCAAGTGAGGGCCCTGAGAAAAGACAGCGTGTACTTCAACTACACTAACAC  
ATTAAAAAAATAAGTATTGGAGGCCAGAGACAGCTACTTCAAGAACATGCT  
AAAAGGAGAGTATCAAAACTGGTTGACCTGGAGGTGACTGACCATCACCGGG  
ATTACTTCGCTGAGTCCATTAGTGGTGTAGTAGCCCTTGGTGGCAGAT  
ATGTACTTTGGTACTGGTACATACATGGCTTACAGAACAGAACAGGCCCTAG  
GGATTCACTATGGATCAGGGGAAGTGGTGTAGTAGGGCAACTTGCTAACCCAT  
AACAAATTGAAGTGGTACATACACTTCTGCTGTACCTACTGCTGAGGGAG  
GAGAGCGTAAAGAAGTGGTCTTACTCTTACACCATCTTACTGTTGACACCCA  
ATCAAATCTGTAATTGTGATCCTACTGATGATTGGGATGTGGTAAAGGCCGAT  
TCAGGGGCCAAGAGTACTTGGGAAATAGACCTCTGTTACAAACAGTAGT  
ACTAATCGTCATAAGGTTAATCATAGCCAGGCGTACACTGAACGACCCACAGCCTGGAG  
GGTAACAATAATGGCAGCACTGAGGGTCACTGAACGTACCCACAGCCTGGAG  
TTGACATCGCTGGCGGTACTGACTATAACCCACTGATGGTAGCTATGTGA  
CAGATTATTTAGATATAAAAATGGTACAGTGCATTCTCAGCTGGTATCTGC  
GGTGTCTGATAAGAACGCTAATATACCTAGGTAGAATCGAGATGCCAGAGG  
TAACTATCCAAACTGGAGGACCAACTTAAACTATTATATTGATCTCAAC  
AACAAATTGTAACGAGGTGGAAGGGTACGTGGCTGGCCTATTGTTGCAATGTG  
TGCCTATCTATTGCTGGTCACAACCTGTGGGCCACTCTTAACCTAATACT  
GATCCTGCCTACCTATGAATTGGTAAATTATACTATCTGAAAAGTGTAGGACT  
GATATAGAAAGAAGTTGGCTAGGGGGATAGACTATACAAGAGTTGACTCCAT  
CTACGACGTTGATGAGAGTGGAGAGGGCGTATATCTTCCATCAAGGCAGA  
AAGCACAGGGAAATTCTATACCTTGCCTTATCAAAGCAACACTGATAA  
GTTGCGTCAGCAGTAAATGGCAGCTAATATACATGAGTTACTAACTTGGACT  
TTATGTACTACATGCACAGGAAAGTATAGAAGAGATCTCAGGAGGTACCAACA  
TAATATCCAGGTTAGTGGCAGCACTCATAGAGCTGAACGTGCTGGCATGGAAAGAA  
GAGGAGAGCAAAGGCTTAAAGAAGTTTATCTATTGCTGGAGGTGAGAAA  
CCTAATAATAAAACATAAGGTAAAGGAATGAGACCGTGGCTTGTACGGGG  
AGGAGGAAGTCTACGGTATGCCAAAGATCATGACTATAATCAAGGCCAGTACA  
CTGAGTAAGAGCAGGCACTGCATAATATGCACTGTATGTGAGGGCCGAGAGTG  
GAAAGGTGGCACCTGCCAAATGTGGACGCCATGGAAAGGCCATAACGTGT  
GGGATGTCGCTAGCAGATTGAGGGTATGTGCAAGCCGATGCCAGGAAAGCATAGGAGGT  
TTGAAATGGACCGGGAAACCTAAGAGTGTGCTGAGTGTAAATAGG  
CTGCATCCTGCTGAGGAAGGTGACTTTGGCAGAGTCGAGCATGTTGGCCT  
AAAAATCACCTACTTGCCTGATGGATGGAAAGGTGTATGATATCACAGAGTG  
GGCTGGATGCCAGCGTGGGAATCTCCCCAGATAACCCACAGACTCCCTGTC  
ACATCTCATTTGGTTACGGATGCCCTTCAGGCAGGAATACAATGGCTTGTAC  
AATATACCGCTAGGGGCAACTATTCTGAGAAACTGCCCCTACTGGCAACTA  
AAGTAAAAATGCTCATGGTAGGCAACCTGGAGAAGAAATTGTAATCTGGAA  
CATCTGGGTGGATCCTAAGGGGGCTGCCGTGTGTAAGAAGATCACAGAGCA  
CGAAAAATGCCACATTAATATACTGGATAAAACTAACCGCATTTCGGGATCAT  
GCCAAGGGGGACTACACCCAGAGCCCCGGTGAGGTTCCCTACGAGCTTACTAA  
AAGTGAGGAGGGTCTGGAGACTGCCTGGCTTACACACACCAAGGCAGGGAT

AAGTTCACTGACCATGTAACCGCCGGAAAAGATCTACTGGTCTGTGACAGCA  
TGGGACGAACTAGAGTGGTTGCCAAAGCAACAACAGGTTGACCGATGAGACA  
GAGTATGGCGTCAAGACTGACTCAGGGTGCCAGACGGTGCCAGATGTTATGT  
GTTAAATCCAGAGGCCGTTAACATATCAGGATCCAAGGGCAGTCGTTCACC  
TCCAAAAGACAGGTGGAGAATTACGTGTCAACCGCATCAGGCACACCGGCT  
TTCTTCGACCTAAAAAACTGAAAGGATGGTCAGGTTGCCTATAATTGAAGCC  
TCCAGCAGGGAGGGTGGTGGCAGAGTCAAAGTAGGAAAGAATGAAGAGTCTA  
AACCTACAAAAATAATGAGTGGAAATCCAGACCGTCTCAAAAACAGAGCAGAC  
CTGACCGAGATGGTCAAGAAGATAACCAGCATGAACAGGGAGACTTCAGA  
GATTACTTGGCAACAGGGCAGGCAAAACACAGAACTCCAAAAGCAGTTA  
TAGAGGAGATAGGAAGACACAAGAGAGTATTAGTCTTATACCATTAAGGGCA  
GCGGCAGAGTCAGTCTACAGTATATGAGATTGAAACACCCAAGCATTCTT  
AACCTAAGGATAGGGACATGAAAGAGGGGACATGGCAACCGGGATAACCT  
ATGCATCATACGGGTACTTCTGCCAAATGCCCTAACCAAAGCTCAGAGCTGCTA  
TGGTAGAATACTCATACATATTCTAGATGAATACCATTTGTGCCACTCTGAACA  
ACTGGCAATTATCGGGAAAGATCCACAGATTTCAGAGAGTATAAGGGTTGCG  
CCATGACTGCCACGCCAGCAGGGTGGTACCCACAGGCTAAAAGCACCCA  
ATAGAGGAATTCAATAGCAGGGTAAAGGAGGATCTGGTAGTCA  
GTTCTTGATATAGCAGGGTAAAGGAGGATCTGGTAGTCA  
GTTGGTTTGTACCAACGAGAACATGGCAGTAGAGGTAGCAAAGAACGTTAA  
AAGCTAAGGGCTATAACTCTGGATACTATTACAGTGGAGAGGATCCAGCCAAT  
CTGAGAGTTGTGACATCACAACTCCCCCTATGTAATCGTGGCTACAAATGCTATT  
GAATCAGGAGTGCACACTACCAGATTGGACACGGTTAGACACGGGTTGAA  
ATGTGAAAAGAGGGTGGGGTACATCAAAGATAACCTTACGTAACAGGCC  
TTAAGAGGATGGCGTGAUTGTGGTGAGCAGGCGCAGCGTAGGGCAGAGT  
AGGTAGAGTGAACCGGGAGGTATTAGGAGCCAGGAAACAGCAACAGGG  
TCAAAGGACTACCACTATGACCTCTGCAGGCACAAAGATAACGGGATTGAGGA  
TGGAAATCAACGTGACGAAATCTTGTAGGGAGATGAATTACGATTGGAGCCTATA  
CGAGGAGGACAGCCTACTAATAACCCAGCTGGAAATACTAAATAATCTACTCAT  
CTCAGAAGACTTGCAGCCGCTGTTAAGAACATAATGCCAGGACTGATCACC  
CAGAGCCAATCCAACCTGCATACAAACAGCTATGAAGTCCAGGTCCCCTG  
TCCCAAAAATAAGGAATGGAGAAGTCACAGACACCTACGAAAATTACTCGTT  
TAAATGCCAGAAAGTTAGGGGAGGATGTGCCGTGTATATCTACGCTACTGAA  
GATGAGGATCTGGCAGTTGACCTCTAGGGCTAGACTGGCCTGATCTGGGAA  
CCAGCAGGTAGTGGAGACTGGTAAAGCACTGAAGCAAGTGGACCGGGTTGCT  
CGGCTGAAATGCCCTACTAGTGGTTATTGGGTATGTGGGTTACCAAGGCTC  
TCTCAAAGAGGCATGCCCCATGATAACAGACATATACCATCGAGGACCGA  
GAATAGAAGACACCAACCCACCTCCAGTATGCACCCAAACGCCATAAAAACCGAT  
GGGACAGAGACTGAACGAAAGAACTGGCGTGGGTGACGTGGAAAAAAATCA  
TGGGAGGCCATTTCAGATTATGCAGCTGGGGACTGGAGTTGTTAAATCCAA  
GCAGAAAAGATAAAACAGCTCTTGTAAAGAAAACCGCAGAACGCCGCAA  
AGGTATGTCCAAAATTCACTGACTCATTAATTGAAAATAAGAAGAAATAAT  
CAGATATGGTTGTGGGGAAACACACAGCACTATACAAAGCATAGCTGCAA  
GAATGGGGCATGAAACAGCTTGTCCACACTAGTGTAAAGTGGTAGCTTT  
GGGGGGGAATCACTGTCAGACCAACGTCAGCAGGGCAGTTGATTAGTG  
TCTATTATGTGATGAAATAAGCCTCTCCCTCCAGGTGACTCCGAGACACAGCAAG  
AAGGGAGGCATTGCGCAAGCCTGTTACCTCCGACTGGCAACCTACACA  
TACAAAACCTGGAATTACCAACATCTCTAAAGTGGTGGAAACAGCCCTGGCT  
TACCTCCCCATGCTACCAACGCTTAAAGTGTGACGGCTGAAACAGCAAG  
AGCGTGGTACTGAGCACCACGATATATAACCTCTCTATAAGGAAG  
GGGAAGAGTGTGGATTGCTGGGTACGGGGATAAGTGCAGCCATGGAAATCC  
TGTCAACAAAACCCAGTATCGGTAGGTATATCTGTGATGTTGGGGTAGGGGCA  
ATCGCTGCGCACACGCTATTGAGTCCAGTGAACAGAAAAGGACCTACTTAT  
GAAGGTGTTGAAAGAACTCTTGGATCAGGCTGCAACAGATGAGCTGGTAA

AAGAAAACCCAGAAAAAATTATAATGGCCTTATTGAAGCAGTCCAGACAATTG  
GTAACCCCCCTGAGACTAATATACCACCTGTATGGGGTTACTACAAAGGTTGGG  
AGGCCAAGGAACTATCTGAGAGGACAGCAGGCAGAAACTTATTCACATTGATA  
ATGTTTGAAGCCTCGAGTTATTAGGGATGGACTCACAAGGGAAAATAAGGAA  
CCTGTCGGAAATTACATTGGATTGTATACGGCCTACACAAGCAAATCAA  
CAGAGGGCTGAAGAAAATGGTACTGGGGGGCCCTGCACCCCTTAGTTGTG  
ACTGGACCCCTAGTGACGAGAGGATCAGATTGCCAACAGACAACACTATTGAGG  
GTAGAAACCAGGTGCCATGTGGTATGAGATGAAAGCTTCAAAATGTAGG  
TGGCAAACCTTACCAAAGTGGAGGAGAGCGGGCTTCCTATGTAGAAAACAGAC  
CTGGTAGGGGACCAGTCACACTACAGAGTCACCAAGTATTACGATGACAACCTC  
AGAGAGATAAAACCACTAGCAAAGTTGGAAGGACAGGTAGAGCACTACTACAA  
AGGGGTACAGCAAAATGACTACAGTAAAGGAAAATGCTTGGCCACTG  
ACAAGTGGGAGGTGGAACATGGTGTCACTAACAGGTTAGCTAAGAGATAACT  
GGGGTCCGGGTTCAATGGTGCATACTTAGGTGACGAGGCCAATACCCGTGCTCT  
AGTGGAGAGGGACTGTGCAACTATAACCAAAACACAGTACAGTTCTAAAAAT  
GAAGAAGGGGTGCGTTCACCTATGACCTGACCATCTCCAATCTGACCAGGC  
TCATCGAACTAGTACACAGGAACAATCTGAAGAGAAGGAAATACCCACCGCT  
ACGGTCACCATGGCTAGCTTACACCTCGTGAATGAAGACGTAGGGACTAT  
AAAACCACTACTAGGAGAGAGATAATCCCCGACCCCTGTAGTTGATATCAATT  
ACAACCAAGGGTGCAGTGGACACGTCAAGGAGTTGGATCACAATAATTGGA  
GGGAAACCCCTGATGACAACGGGAGTGACACCTGTCTGGAAAAAGTAGAGCCT  
GACGCCAGCGACAACCAAAACTCGGTGAAGATCGGGTGGATGAGGGTAATT  
CCCAGGGCCTGGAATACAGACACATACACTAACAGAAGAAATACACAACAGGG  
ATGCGAGGCCCTCATCATGATCCTGGCTCAAGGAATTCCATATCAAATAGGG  
CAAAGACTGCTAGAAATATAATCTGTACACAGGAAATGACCCCCAGGGAAATA  
CGAGACTTGTGCTGCAGGGCGATGTTAGTAGCTGACTGAGGGATGTCGA  
CCCTGAGCTGTCGAAATGGTCATTCAAGGGGACTTTTAGATAGGGAGG  
CCCTGGAGGCTCTAAGTCTCGGGCAACCTAACCGAAGCAGGTACCAAGGAA  
GCTGTTAGGAATTGATAGAACAGAAAAAGATGTGGAGATCCCTAACTGGTT  
GCATCAGATGACCCAGTATTCTGGAAGTGGCCTAAAAAATGATAAGTACTAC  
TTAGTAGGAGATGTTGGAGAGCTAAAGATCAAGCTAAAGCACTGGGGCAC  
GGATCAGACAAGAATTATAAGGAGGTAGGCTCAAGGACGTATGCCATGAAGC  
TATCTAGCTGGTCTCAAGGCATCAAACAAACAGATGAGTTAATCCTACTGT  
TTGAGGAATTGTTGCTACGGTGCCACCTGCAACTAAGAGCAATAAGGGGCAC  
ATGGCATCAGCTTACCAATTGGCACAGGGTAACTGGGAGGCCCTGGTTGCGG  
GGTCACCTAGGTACAATACCAGCCAGAAGGGTGAAGATAACCCATATGAAG  
CTTACCTGAAGTTGAAAGATTCTAGAGAAGAAGAGAAGAAACCTAGGGTT  
AAGGATAACAGTAATAAGAGAGCACAACAAATGGTACTTAAAAAAATAAGGTT  
CAAGGAAACCTCAACACCAAGAAAATGCTCAACCCGGGAAACTATCTGAACA  
GTTGGACAGGGAGGGCGCAAGAGGAACATCTACAACCACAGATTGGTACT  
ATAATGTCAAGTGCAGGCATAAGGCTGGAGAAATTGCCAATAGTGAGGGCCA  
AACCGACACCAAAACCTTCTGAGGCAATAAGAGATAAGAGATAAGAGTG  
AAAACCGGCAAAATCCAGAATTGCAACAAATTGTTGGAGATTCCACACGA  
TAGCCCCAACCCACCTGAAACACACCTACGGTGAAGGTGACGTGGGAGCAACT  
GAGGCGGGATAAAATAGAAAGGGGGCAGCAGGCTCTGGAGAGAAGAAGAAC  
TCGGAGAAGTATTGGATTGAGAAAGATAAAATATTATGAAACTGCAATACCAAAATGA  
CTGAAGGCCGGAGAAAGATAAAATATTATGAAACTGCAATACCAAAATGA  
GAAGAGAGATGTCAGTGACTGGCAGGCAGGGGACCTGGTGGTTGAGAAG  
AGGCCAAGAGTTATCCAATACCTGAGCAAGCAAGACAAGGCTAGCCATCACTAA  
GGTCATGTATAACTGGGTGAAACAGCAGGCCGTTGTGATTCCAGGATATGAAG  
GAAAGACCCCTGTTCAACATCTTGATAAAAGTGAAGAAAGGAATGGGACTCGT  
TCAATGAGCCAGTGGCCGTAAGTTGACACCAAAGCCTGGGACACTCAAGTG  
ACTAGTAAGGATCTGCAACTTATTGGAGAAATCCAGAAATATTACTATAAGAAG  
GAGTGGCACAAGTTCATTGACACCACATGCCGACCACATGACAGAAGTACCAAGT

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FIGURE 19-5

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|           |  |        |     |
|-----------|--|--------|-----|
|           | AATTCTGCTCATGACGGCCGTGGCGTCAGCAGCTGAAGGTTGGGTAAACACTCCGCCCTT           | AGGCCA |     |
| 3H3Bfrag  | AATTCTGCTCATGACGGCCGTGGCGTCAGCAGCTGAAGGTTGGGTAAACACTCCGCCCTT           | AGGCCA | 70  |
| 1.1.4 seq | AATTCTGCTCATGACGGCCGTGGCGTCAGCAGCTGAAGGTTGGGTAAACACTCCGCCCTT           | AGGCCA | 70  |
| 1.2.3 seq | AATTCTGCTCATGACGGCCGTGGCGTCAGCAGCTGAAGGTTGGGTAAACACTCCGCCCTT           | AGGCCA | 70  |
| 6.2.2 seq | AATTCTGCTCATGACGGCCGTGGCGTCAGCAGCTGAAGGTTGGGTAAACACTCCGCCCTT           | AGGCCA | 70  |
| 6.1.4 seq | AATTCTGCTCATGACGGCCGTGGCGTCAGCAGCTGAAGGTTGGGTAAACACTCCGCCCTT           | AGGCCA | 70  |
|           | <hr/>  |        |     |
|           | TTTCCCTGTT           |        |     |
| 3H3Bfrag  | TTTCCCTGTT           |        | 140 |
| 1.1.4 seq | TTTCCCTGTT             |        | 109 |
| 1.2.3 seq | TTTCCCTGTT               |        | 102 |
| 6.2.2 seq | TTTCCCTGTT                   |        | 99  |
| 6.1.4 seq | TTTCCCTGTT                       |        | 84  |
|           | <hr/>  |        |     |
|           | -----CCTTOCTCTTTTTTCCCTTCTTCCCTCCCTCTTAATG                             |        |     |
| 3H3Bfrag  | -----CCTTOCTCTTTTTTCCCTTCTTCCCTCCCTCTTAATG                             |        | 210 |
| 1.1.4 seq | -----CCTTOCTCTTTTTTCCCTTCTTCCCTCCCTCTTAATG                             |        | 149 |
| 1.2.3 seq | -----CCTTOCTCTTTTTTCCCTTCTTCCCTCCCTCTTAATG                             |        | 142 |
| 6.2.2 seq | -----CCTTOCTCTTTTTTCCCTTCTTCCCTCCCTCTTAATG                             |        | 140 |
| 6.1.4 seq | -----CCTTOCTCTTTTTTCCCTTCTTCCCTCCCTCTTAATG                             |        | 125 |
|           | <hr/>  |        |     |
|           | GTCGGCTCCATCTAGCCCTAGTCACGGCTAGCTGTGAAAGGTTGGTGAGCCGCATGACTGCAGAGAGTGC |        |     |
| 3H3Bfrag  | GTCGGCTCCATCTAGCCCTAGTCACGGCTAGCTGTGAAAGGTTGGTGAGCCGCATGACTGCAGAGAGTGC |        | 280 |
| 1.1.4 seq | GTCGGCTCCATCTAGCCCTAGTCACGGCTAGCTGTGAAAGGTTGGTGAGCCGCATGACTGCAGAGAGTGC |        | 219 |
| 1.2.3 seq | GTCGGCTCCATCTAGCCCTAGTCACGGCTAGCTGTGAAAGGTTGGTGAGCCGCATGACTGCAGAGAGTGC |        | 212 |
| 6.2.2 seq | GTCGGCTCCATCTAGCCCTAGTCACGGCTAGCTGTGAAAGGTTGGTGAGCCGCATGACTGCAGAGAGTGC |        | 210 |
| 6.1.4 seq | GTCGGCTCCATCTAGCCCTAGTCACGGCTAGCTGTGAAAGGTTGGTGAGCCGCATGACTGCAGAGAGTGC |        | 195 |
|           | <hr/>  |        |     |
|           | TGATACTGGCTCTCTGCAGATCATGTCCCCGGCGTGGCGTCAGCAGCTGAGACAAAAATGTATATAT    |        |     |
| 3H3Bfrag  | TGATACTGGCTCTCTGCAGATCATGTCCCCGGCGTGGCGTCAGCAGCTGAGACAAAAATGTATATAT    |        | 350 |
| 1.1.4 seq | TGATACTGGCTCTCTGCAGATCATGTCCCCGGCGTGGCGTCAGCAGCTGAGACAAAAATGTATATAT    |        | 289 |
| 1.2.3 seq | TGATACTGGCTCTCTGCAGATCATGTCCCCGGCGTGGCGTCAGCAGCTGAGACAAAAATGTATATAT    |        | 282 |
| 6.2.2 seq | TGATACTGGCTCTCTGCAGATCATGTCCCCGGCGTGGCGTCAGCAGCTGAGACAAAAATGTATATAT    |        | 280 |
| 6.1.4 seq | TGATACTGGCTCTCTGCAGATCATGTCCCCGGCGTGGCGTCAGCAGCTGAGACAAAAATGTATATAT    |        | 265 |
|           | <hr/>  |        |     |
|           | TGTAAATAAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT                  |        |     |
| 3H3Bfrag  | TGTAAATAAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT                  |        | 402 |
| 1.1.4 seq | TGTAAATAAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT                  |        | 341 |
| 1.2.3 seq | TGTAAATAAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT                  |        | 334 |
| 6.2.2 seq | TGTAAATAAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT                  |        | 332 |
| 6.1.4 seq | TGTAAATAAAATTAATCCATGTACATAGTGTATATAAATATAGTTGGGACCGT                  |        | 317 |

FIGURE 20

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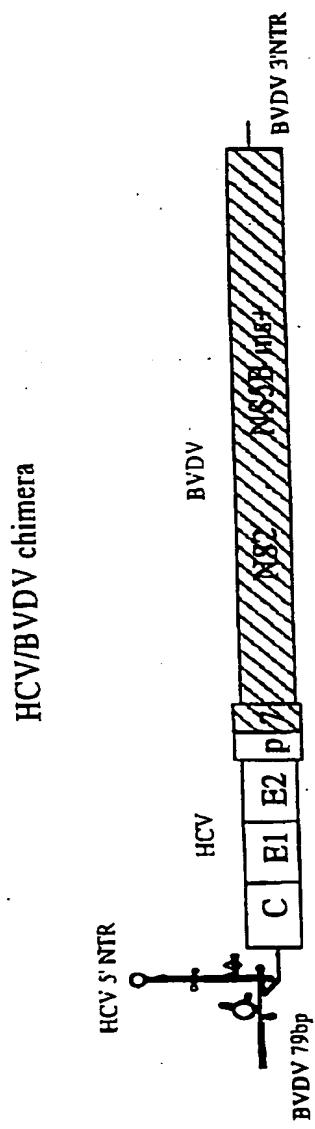


FIGURE 21

GTatcagagaatttagaaaaggcactcgatacgtattggcaattaaaaataataattaggccctagtgcatggcacgtgccagccccct  
gatggggggcgacactccaccatgaatcactcccccgtggagggactactgtctcgcagaaaggcgttagccatggcgtagatgag  
tgtcggtcgaggccctccggagggccatagtggctcgggaccggtagtgcggatccggatccggatccggacgac  
cggttccttcttggataaaccgcgtcaatgcctggagattggcgatccggatccggatccggatccggacgac  
aggccttggtaactgcctatagggtgtcgagtgccccggggctgttagaccgtgcaccATGAGCACGAATC  
CTAAACCTCAAAGAAAAACCAACGTAAACACCAACCCTCGCCCACAGGACGTC  
AAGTTCCGGGTGGCGGTCAAGATCGTTGGTGGAGTTACTTGTTGCCGCCAG  
GGGCCTAGATTGGGTGTGCGCGCGACGGAGGAAGACTTCCGAGCGGTGCGCAA  
CCTCGAGGTAGACGTCAGCCTATCCCCAAGGCACGTGCGCCGAGGGCAGGA  
CCTGGGCTCAGCCCCGGTACCCCTGGCCCTCATGGCAATGAGGGTTGCGGG  
TGGGCGGGATGGCTCCTGTCTCCCGTGGCTCTCGGCCATAGCTGGGGCCAC  
AGACCCCCGGCGTAGGTGCGCAATTGGTAAGGTATCGATAACCTTAAGT  
GCGGCTTCGCCACCTCATGGGGTACATACCGCTCGTGGGCCCTTGGGA  
GGCGCTGCCAGGGCCCTGGCGATGGCGTCCGGGTTCTGGAAAGACGGCGTGA  
ACTATGCAACAGGGAACCTTCCTGGTGTCTTCTCATCTTCCTCTGGGCC  
GCTCTTGCCTGACCGTGCCCTCAGCCTACCAAGTGCAGCAATTCTCGGG  
GCTTACCATGTCACCAATGATTGCCCTAACTCGAGTATTGTGACGAGGGC  
CGATGCCATCCTGCACACTCCGGGTGTGTCCTTGCCTCGCAGGGTAACG  
CCTCGAGGTGTTGGTGGCGGTGACCCCCACGGTGGCACCCAGGGACGGCAA  
ACTCCCCACAACGCACTCGACGTATCGATCTGCTGTCGGGAGCGCCA  
CCCTGCTCGGCCCTCACGTGGGGGACCTGTGCGGGTCTGCTTCTGTTG  
GTCAACTGTTACCTCTCCCAGGCGCCACTGGACGACGCAAGACTGCAATT  
GTTCTATCTATCCGGCCATATAACGGGTATCGCATGGCATGGGATATGATGA  
TGAACGGTCCCCCTACGGCAGCGTGGTGGTAGCTCAGTGTCCGGATCCCA  
CAAGCCATCATGGACATGATCGCTGGTGTCACTGGGGAGTCTGGGGCAT  
AGCGTATTCTCCATGGGGAACTGGCGAAGGTCTGGTAGTGTGCTGTC  
TATTGCGGGCGTCACGCCAAACCCACGTACCGGGGGAAAGTGCCTGGCG  
CACCAAGGCTGGCTTGTGGTCTCCTACACCAGGCGCCAAGCAGAACATCC  
AACTGATCAACACCAACGGCAGTTGGCACATCAATAGCACGGCCTGAACTGC  
AATGAAAGCCTAACACCGGCTGGTAGCAGGGCTTCTATCAGCACAAATT  
AACTCTCAGGCTGTCTGAGAGGTTGCCAGCTGCCAGCCTTACCGATTT  
GCCCAAGGGCTGGGTCTATCAGTTATGCCAACGGAAAGCGGCCCTGACGAAC  
GCCCTACTGCTGGCACTACCCCTCAAGACCTGTGGCATTTGTGCCGCCAAAG  
AGCGTGTGTGGCCGGTATATTGCTTACCTCCAGCCCCGTGGTGGGGAAAC  
GACCGACAGGTGGCGGCCACCTACAGCTGGGTGCAAATGATACGGAT  
GTCTTGTCTTAAACACACCAGGCGCCAGCTGGCAATTGGTTGGTTGAC  
TGGATGAACTCAACTGGATTACCAAAAGTGTGCGGAGGCCCTTGTGTC  
CGGAGGGTGGCAACACACCTTGTCTGCCCAACTGATTGTTCCGCAAGC  
ATCCGGAAGCCACATACTCTCGGTGCCGCTCCGGTCCCTGGATTACACCCAGG  
TGCATGGTCGACTACCCGTATAAGGCTTGGCACTATCCTGTACCATCAATTAC  
ACCATATTCAAAGTCAGGATGTACGTGGAGGGGTGAGCACAGGCTGGAAAG  
CGGCCTGCAACTGGACGCCGGCGAACGCTGTGATCTGGAAAGACAGGGACAG  
GTCCGAGCTCAGCCATTGCTGTCCACCACACAGTGGCAGGTCTTCCGT  
GTTCTTCAAGCACCTGCCAGCCTGTCCACCAGGCCATCCACCTCCACCAAGA  
ACATTGTGGACGTGAGTACTGTACGGGTAGGGTCAAGCAGTGCCTGCTGG  
GCCATTAAGTGGAGTACGTGTTCTCTGTGTTCTGCTTGCAGACGCCGCG  
GTCTGCTCTGCTTGTGGATGATGTTACTCATATCCCAAGCGGAGGCCGTTG  
GAGAACCTCGTAATACTCAATGCAAGCATCCCTGGCCGGACGCACGGTCTTGT  
GTCCTCTCGTGTCTTGTGCTTGCCTGGTATCTGAAGGGTAGGTAGGTGGGGC

FIGURE 22-1

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CGGAGCGGTCTACGCCCTCACGGGAAGTGGGTCTACTCTTATACCACATCTT  
AGTGGTACACCCAATCAAATCTGTAATTGTGATCCTACTGATGATTGGGATGT  
GGTAAAGGCCGATTCAAGGGGCAAGAGTACTTGGGAAAATAGACCTCTGTT  
TTACAACAGTAGTACTAATCGTCATAGGTTAACATAGCTAGGCCTGACCCAA  
CTATAGTGCCTGGTAACAATAATGGCAGCACTGAGGGTCACTGAACGTGACC  
CACCAAGCCTGGAGTTGACATCGCTGTGGCGGTATGACTATAACCCTACTGAT  
GGTTAGCTATGTGACAGATTATTTAGATATAAAAAATGGTACAGTGCATTCTC  
AGCCTGGTATCTGCGGTCTTGATAAGAAGCCTAATATACCTAGGTAGAATC  
GAGATGCCAGAGGTAACTATCCAAACTGGAGACCAACTAACTTAAACTATTA  
TATTTGATCTAACAAACAATTGTAACGAGGTGGAAGGGTACGTGGCTGGCCTA  
TTGTTGCAATGTGTCCTATCTTATTGCTGGTACAACCTGTGGCCACTTCT  
TAACCCCTAACACTGATCCTGCCTACCTATGAATTGGTAAATTATACTATCTGAA  
AACTGTTAGGACTGATACAGAAAGAAGTGGCTAGGGGGATAGACTATACAA  
GAGTTGACTCCATCTACGACGTTGATGAGAGTGGAGAGGGCGTATATCTTTTC  
CATCAAGGCAGAAAGCACAGGGAAATTCTATACTCTTGGCCCTTATCAAAG  
CAACACTGATAAGTGCCTCAGCAGTAAATGGCAGCTAATATACATGAGTTACT  
TAACCTTGGACTTATGTAACATGCACAGGAAAGTTATAGAAGAGATCTCAG  
GAGGTACCAACATAATATCCAGGTTAGTGGCAGCACTCATAGAGCTGAACCTGG  
TCCATGGAAGAAGAGGGAGGCAAGGCTTAAAGAAGTTTTATCTATTGCTGG  
AAGGTTGAGAAACCTAACATAAAACATAAGGTAAGGAATGAGACCGTGGCTT  
CTTGGTACGGGAGGAGGAAGTCTACGGTATGCCAACAGATCATGACTATAATC  
AAGGCCAGTACACTGAGTAAGAGCAGGCACTGCATAATATGCACTGTATGTGA  
GGGCCAGAGTGGAAAGGTGGCACCTGCCAAATGTGGACGCCATGGGAAG  
CCGATAACGTGTTGGATGTCCTAGCAGATTGAAAGAAAGACACTATAAAAG  
AATCTTATAAGGGAAGGCAACTTGTGAGGGTATGTGCAGCCGATGCCAGGGAA  
AGCATAGGAGGTTGAAATGGACCGGGAACCTAACAGTGCAGATACTGTGCT  
GAGTGTAAATAGGCTGCATCTGCTGAGGAAGGTGACTTTGGCAGAGTCGAG  
CATGTTGGCCTCAAAATACCTACTTGTGCTGATGGATGGAAAGGTGTATGA  
TATCACAGAGTGGCTGGATGCCAGCGTGTGGAAATCTCCCCAGATACCCACA  
GAGTCCCTTGTACATCTCATTTGGTACGGATGCCCTTCAGGCAGGAATACA  
ATGGCTTGTACAATATACCGCTAGGGGCAACTATTCTGAGAAACTTGCCCCG  
TACTGGCAACTAAAGTAAAATGCTCATGGTAGGCAACCTGGAGAAGAAATT  
GGTAATCTGGAACATCTGGGTGGATCCTAACGGGGCTGCCGTGTGAAGAA  
GATCACAGAGCACGAAAATGCCACATTAATATACTGGATAAAACTAACCGCATT  
TTTCGGGATCATGCCAACGGGGACTACACCCAGAGCCCCGGTGGAGACTGCC  
CGAGCTTACTAAAAGTGAGGAGGGGTCTGGAGACTGCCCTGGCTACACACAC  
CAAGGCGGGATAAGTTCAGTCGACCATGTAACGCCGAAAAGATCTACTGGT  
CTGTGACAGCATGGGACGAACTAGAGTGGTTGCCAACAGCAACACAGGTTGA  
CCGATGAGACAGAGTATGGCGTCAAGACTGACTCAGGGTGCCAGACGGTGC  
CAGATGTTATGTGTTAAATCCAGAGGCCGTTAACATATCAGGATCAGGGGG  
CAGTCGTTCACCTCCAAAAGACAGGTGGAGAATTCACTGTTGTACCGCATCA  
GGCACACCGGCTTCTCGACCTAAAAAAACTTGAAGAGTGGTCAGGCTTGCCT  
ATATTGAAAGCCTCCAGCGGGAGGGTGGTGGCAGAGTCAAAGTAGGGAAAGA  
ATGAAGAGTCTAAACCTAACAAAATAATGAGTGGAAATCCAGACCGCTCAAAAAA  
ACAGAGCAGACCTGACCGAGATGGTCAAGAAGATAACCCAGCATGAAACAGGGG  
AGACTTCAAGCAGATTACTTGTGCAACAGGGCAGGCAAAACACAGAACTCC  
CAAAGCAGTTATAGAGGAGATAGGAAGACACAAGAGAGTATTAGTTCTTATA  
CCATTAAGGGCAGCGGAGACTCAGTCTACCAAGTATATGAGATTGAAACACCC  
AAGCATCTTTAACCTAACGGATAGGGACATGAAAGAGGGGACATGGCAA  
CCGGGATAACCTATGCATCATACGGGTACTCTGCCAACATGCCCTAACCAAAGC  
TCAGAGCTGCTATGGTAGAATACTCATACATATTCTAGATGAATACCATTGTGC  
CACTCCTGAACAACGGCAATTATCGGGAAAGATCCACAGATTTCAGAGAGTAT  
AAGGGTTGTCGCCATGACTGCCACGCCAGCAGGGTGGTACCAACACAGGT  
CAAAGCACCAATAGAGGAATTGATAGCCCCGAGGTAATGAAAGGGGAGG

FIGURE 22-2

ATCTTGGTAGTCAGTCCTGATATAGCAGGGTAAAAAATACCACTGGATGAGA  
TGAAAGGCAATATGTTGGTTTGACCAACGAGAAACATGGCAGTAGAGGTA  
GCAAAGAAGCTAAAAGCTAAGGGCTATAACTCTGGATACTATTACAGTGGAGA  
GGATCCAGCCAATCTGAGAGTTGTGACATACAATCCCCCTATGTAATCGTGGC  
TACAAATGCTATTGAATCAGGAGTGAACACTACCAGATTGGACACGGTTATAGA  
CACGGGGTTGAAATGTAAAAGAGGGTGGAGGGTATCATCAAAGATAACCCTCA  
TCGTAACAGGCCTAAGAGGATGCCGTGACTGTGGGTGAGCAGGCGAGCG  
TAGGGGCAGAGTAGGTAGAGTGAAACCCGGGAGGTATTATAGGAGGCCAGGAA  
ACAGCAACAGGGTCAAAGGACTACCAACTATGACCTTGCAGGCACAAAGATA  
CGGGATTGAGGTGGAATCAACGTGACGAAATCCTTAGGGAGATGAATTACG  
ATTGGAGCCTATACGAGGAGGACAGCCTACTAATAACCCAGCTGAAATACTA  
AATAATCTACTCATCTCAGAAGACTTGCCAGCCGCTGTTAAGAACATAATGGCC  
AGGACTGATCACCCAGAGCCAATCCAACCTGCATACAACAGCTATGAAGTCCA  
GGTCCCAGGTCTTATTCCAAAATAAGGAATGGAGAAGTCACAGACACCTACG  
AAAATTACTCGTTCTAAATGCCAGAAAGTTAGGGAGGATGTGCCGTGATA  
TCTACGCTACTGAAGATGAGGATCTGGCAGITGACCTTCTAGGGCTAGACTGG  
CCTGATCCTGGAAACCAGCAGGTAGTGGAGACTGGTAAAGCACTGAAGCAAGT  
GACCGGGTTGTCCTCGGCTGAAAATGCCCTACTAGTGGCTTATTGGGTATGT  
GGGTTACAGGCTCTCAAAGAGGGCATGTCCTAAATGATAACAGACATATATAC  
CATCGAGGACCAGAGACTAGAACAGACACCACCCACTCCAGTATGCACCCACG  
CCATAAAAACCGATGGGACAGAGACTGAAGAACCTCCAGTATGCACCCACG  
CGTGGAAAAAAATCATGGGAGGCCATTTCAGATTATGCAAGCTGGGGACTGGAGT  
TTGTTAAATCCCAAGCAGAAAAGATAAAAACAGCTCTTGTAAAGAAAACG  
CAGAAGCCGAAAAGGGTATGTCCAAAATTCAATTGACTCATTAATTGAAAATA  
AAGAAGAAAATAATCAGATATGGTTGTGGGAACACACACAGCACTATACAAA  
AGCATAGCTGCAAGACTGGGCACTGAAACACGCTTGCACACTAGTGTAAA  
GTGGCTAGCTTGGAGGGAAATCAGTGTCAAGACCGTCAAGCAGCGGGCA  
GTTGATTAGTGGCTATTATGTGATGAATAAGCCTCCTCCAGGTGACTCC  
GAGACACAGCAAGAAGGGAGGCGATTGTCGCAAGCCTGTTCATCTCCGCACT  
GGCAACCTACACATACAAAACCTGGAATTACCAACATCTCTAAAGTGGTGG  
ACCAGCCCTGGCTTACCTCCCTATGCTACCAGCGCATTAAAATGTCACCC  
AACCGCGCTGGAGAGCGTGGTGAATCTGAGCACACGATATATAACATACC  
TCTCTATAAGGAAGGGAAAGAGTGTGATGGATTGCTGGTACGGGATAAGTGC  
AGCCATGGAAATCTGTACAAAACCCAGTATCGTAGGTATATCTGTGATGTT  
GGGGGTAGGGGCAATCGTGCACAAACGCTATTGAGTCCAGTGAACAGAAA  
AGGACCTACTTATGAAGGTGTTGAAAGAACCTCTGGATCAGGTGCAACA  
GATGAGCTGGTAAAAGAAAACCCAGAAAAAATTATAATGGCCTTATTGAAGCA  
GTCCAGACAATTGTAACCCCTGAGACTAATATACCACCTGTATGGGTTAC  
TACAAAGGTGGAGGCCAAGGAACATCTGAGAGGACAGCAGGAGAAACT  
TATTCACTTGTATAATGTTGAAGCCTCGAGTTATTAGGGATGGACTCACAAG  
GAAAATAAGGAACCTGTCCGAAATTACATTGGATTGATATAACGGCTAC  
ACAAGCAAATCAACAGAGGGCTGAAGAAAATGGTACTGGGGTGGGCCCTGC  
ACCCCTTACTGTTGACTGGACCCCTAGTGCAGAGAGGATCAGATTGCCAACAG  
ACAACATTTGAGGGTAGAAAACCAAGGTGCCATGTGGCTATGAGATGAAAGCT  
TTCAAAATGTAGGTGGCAAACCTACCAAAGTGGAGGAGAGCGGGCCCTTCT  
ATGTAGAAACAGACCTGGTAGGGGACCGACTCAACTACAGAGTCACCAAGTATT  
ACGATGACAACCTCAGAGAGATAAAACCAAGTAGCAAAGTGGAAAGGACAGGTA  
GAGCACTACTACAAAGGGTACAGCAAACATTGACTACAGTAAAGGAAAAAAT  
GCTCTGGCCACTGACAAGTGGAGGTGGACATGGTGTATAACCAGGTTAG  
CTAAGAGATAACTGGGTCGGGTTCAATGGTGCATACTTAGGTGACGAGGCC  
AATCACCCTGCTAGTGGAGAGGGACTGTGCAACTATAACCAAAACACAGT  
ACAGTTCTAAAATGAAGAAGGGTGTGCGTTACCTATGACCTGACCATCTC  
CAATCTGACCAAGGCTACGACTAGTACACAGGAACAATCTGAAGAGAAGG  
AAATACCCACCGCTACGGTCACCACATGGCTAGCTTACACCTCGTGAATGAAG

FIGURE 22-3

ACGTAGGGACTATAAAACCAGTACTAGGAGAGAGAGTAATCCCCGACCCCTGTA  
GTTGATATCAATTACAACCAGAGGTGCAAGTGGACACGTACAGAGGTTGGGAT  
CACAATAATTGGAAGGGAAACCCCTGATGACAACCGGGAGTGACACCTGTCTTGG  
AAAAAGTAGAGCCTGACGCCAGCGACAACCAAACCTCGGTGAAGAGTCGGGTTG  
GATGAGGTAATTACCCAGGGCCTGGAATACAGACACATAACTAACAGAAGA  
AATACACAAACAGGGATGCGAGGCCTTCATCATGATCCTGGCTCAAGGAATT  
CCATATCAAATAGGGCAAAGACTGCTAGAAATATAATCTGTACACAGGAAATG  
ACCCCAGGAAATACGAGACTTGATGGCTGCAGGGCGATGTTAGTAGTAGCA  
CTGAGGGATGTCGACCCCTGAGCTGTCGAAATGGTCGATTTCAAGGGGACTTT  
TTTATAGGGAGGCCCTGGAGGCTCTAAGTCTCGGGCAACCTAAACCGAAGC  
AGGTTACCAAGGAAGCTGTTAGGAATTGATAGAACAGAAAAAGATGTGGAG  
ATCCCTAATCGGTTGCATCAGATGACCCAGTATTCCTGGAAGTGGCCTTAAAAA  
AATGATAAGTACTACTTAGGAGATGTTGGAGAGCTAAAAGATCAAGCTAAA  
GCACTTGGGCCACGGATCAGACAAGAATTATAAAGGAGGTAGGCTCAAGGA  
CGTATGCCATGAAGCTATCTAGCTGGTTCCTCAAGGCATCAAACAAACAGATGA  
GTTTAACTCCACTGTTGAGGAATTGTCGACGGTGCACAGGGTAACGGGAG  
GCAATAAGGGCACATGGCATCAGCTTACCAATTGGCACAGGGTAACGGGAG  
CCCCTCGGTTGCGGGTGCACCTAGGTACAATACCAGCCAGAAGGGTGAAGAT  
ACACCCATATGAAGCTTACCTGAAGTTGAAGATTTCATAGAAGAAGAAGAGAA  
GAAACCTAGGGTTAAGGATACAGTAATAAGAGAGCACAAACAAATGGATACITA  
AAAAAATAAGGTTCAAGGAAACCTCAACACCAAGAAAATGCTCAACCCAGGG  
AAACTATCTGAACAGTTGGACAGGGAGGGCGCAAGAGGAACATCTACAACCA  
CCAGATTGGTACTATAATGTCAAGTGCAGGCATAAGGCTGGAGAAATTGCCAA  
TAGTGAGGGCCAAACCGACACAAAACCTTTCATGAGGCAATAAGAGATAAG  
ATAGACAAGAGTGAAAACCGGAAAATCCAGAATTGCAACAACAAATTGTTGGA  
GATTTCCACACGATAGCCAACCCACCTGAAACACACCTACGGTGAGGTGA  
CGTGGGAGCAACTTGAGGCAGGGGTAAATAGAAAGGGGGCAGCAGGCTTCCT  
GGAGAAGAAGAACATGGAGAAGTATTGGATTCAAGAAAAGCACCTGGTAGAAC  
AATTGGTCAGGGATCTGAAGGCCGGAGAAAGATAAAATATTATGAAACTGCA  
ATACCAAAAATGAGAAGAGAGATGTCAGTGTACTGGCAGGCAGGGGACC  
TGGTGGTTGAGAAGAGGCCAAGAGTTATCCAATACCCCTGAAGCCAAGACAAGG  
CTAGCCATCACTAAGGTCACTGTATAACTGGTGAACACAGCAGGCCGTTGTGATT  
CCAGGATATGAAGGAAAGACCCCTGTTCAACATCTTGATAAAGTGAGAAAG  
GAATGGACTCGTCAATGAGCCAGTGGCCGTAAGTTTGACACCAAAGCCTG  
GGACACTCAAGTGACTAGTAAGGATCTGCAACTTATTGGAGAAATCCAGAAATA  
TTACTATAAGAAGGAGTGGCACAAGTTCACTGACACCACCGACCATGAC  
AGAAGTACCAAGTATAACAGCAGATGGTAAGTATATAAGAAATGGCAGA  
GAGGGAGCGGCCAGCCAGACACAAGTGCTGGCAACAGCATGTTAAATGTCCT  
GACAATGATGTACGGCTCTGCGAAAGCAGCAGGGGTACCGTACAAGAGTTCA  
ACAGGGTGGCAAGGATCCACGTCTGTGGGGATGATGGCTTCAATAACTGAA  
AAAGGGTTAGGGCTGAAATTGCTAACAAAGGAGTCAGAATTCTTCATGAAGC  
AGGCAAACCTCAGAAGATAACGGAAGGGAAAAGATGAAAGTTGCTATAGAT  
TTGAGGATATAGAGTTCTCTCATACCCAGTCCTGTTAGGTGGCCGACA  
ACACCGATAGTCACATGGCCGGAGAGACACCGCTGTGATACTATCAAAGATG  
GCAACAAAGATTGATTCAAGTGGAGAGAGGGTACACAGCATATGAAAAAGC  
GGTAGCCTTCAGTTCTGCTGATGTTCTCTGAAACCCGCTTGTAGGAGGAT  
TTGCTGTTGGCTTCTGCAACAGCCAGAGACAGACCCATCAAACATGCCAC  
TTATTATTACAAAGGTGATCCAATAGGGGCTATAAAGATGTAATAGGTGGAA  
TCTAAGTGAAGTGAAGAGAACAGGCTTGAGAAATTGGCAAATCTAAACCTAAG  
CCTGTCACGTTGGGGTCTGGACTAACGACACACAAGCAAAGAATAATTCAAG  
ACTGTGTTGCCATTGGGAAAGAAGAGGGCAACTGGCTAGTTAAGCCCAGAGG  
CTGATATCCAGCAAAACTGGCACTTATACATACCTGATAAAGGCTTACATTAC  
AAGGAAAGCATTATGAGCAACTGCAGCTAAGAACAGAGACAAACCCGGTCATG  
GGGTTGGACTGAGAGATAACAGTTAGGTCCCAGTCATGCTGAG

FIGURE 22-4

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AAGGTTGAAAATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGA~~gacaaaatgtat~~  
~~atattgtaaaataaattaatccatgtacatagtgtatataaaatatagttgtggaccgtccacctaagaagacgacgcacgcacag~~  
~~ctaaacagtagtcaagatttatcacctaagataaacactacatttaatgcacacagcacttttagctgtatgaggatacgccccacgtctatag~~  
~~ttggacttaggaaagacctctaacagcccc~~

FIGURE 22-5

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HCV/BVDV chimera with selectable marker

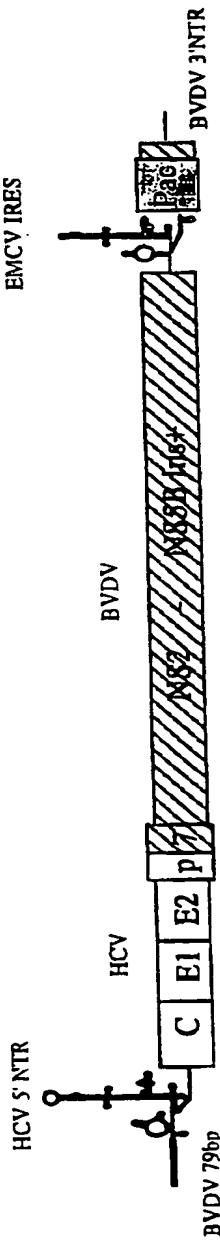


FIGURE 23

Gtatacgagaattagaaaaggcactcgatatacgtaatggcaattaaaaataataattggcttaggtacatggcacgtgccagccccct  
gatgggggcacactccaccatgaatcacccctgtgaggaactactgtctcacgcagaaagcgtctagccatggcgttagttag  
tgtcgtcagccctcaggacccccctccggagagccatagtggctcggaacggtagtacaccggaaatggcaggacgac  
cgggccttcgtgataaaaccgcataitgcctggagattttggcgtagcccgcaagactgttagccgagtagtgtgggtcgca  
aggccgtggtaactgcctgatagggtgtcgagtgccccggagggtctgttagaccgtgcacc.ATGAGCACGAATC  
CTAAACCTCAAAGAAAAACCAACGTAACACCAACCCTCGCCACAGGACGTC  
AAGTTCCGGGTGGCGTCAGATCGTGGTAGTTACTTGTGCCCCAG  
GGGCCCTAGATTGGGTGTGCGCGACGAGGAAGACTTCCCAGCGGTCGCAA  
CCTCGAGGTAGACGTCAGCTATCCCAAGGCACGTGCGCCCGAGGGCAGGA  
CCTGGGCTCAGCCGGGTACCCCTGGCCCCCTCATGGCAATGAGGGTTGCGGG  
TGGGCGGGATGGCTCCTGTCTCCCGTGGCTCGCCTAGCTGGGCCCCAC  
AGACCCCCGGCGTAGGTCGCGCAATTGGGTAAAGGTATCGATAACCTTACGT  
GCGGCTTCGCGACCTCATGGGTACATACCGCTCGCGCCCTTGG  
GGCGCTGCCAGGGCCCTGGCGATGGCTCCGGTTCTGGAAGACGGCGTGA  
ACTATGCAACAGGGAACCTCCTGGTGTCTTCTATCTCCTCTGGCC  
GCTCTCTGCGCTGACCGTGGCGCTTCAGCTACCAAGTGCGCAATTCTCGGG  
GCTTACCATGTCACCAATGATTGCCCTAACCGCTCGCGCCCTTGG  
CGATGCCATCTGCACACTCCGGGTGTGCGCCCTGCGTGCAGGGTAACG  
CCTCGAGGTGTTGGGTGGCGTACCCCCACGGTGGCCACCAGGGACGGCAA  
ACTCCCCACAACGCAAGCTCGACGTATCGATCTGCTTGTGCGGGAGCGCCA  
CCCTCTGCTCGCCCTACGTGGGGGACCTGTGCGGGTCTGTCTTCTTGTG  
GTCAACTGTTACCTCTCCAGGCGCCACTGGACGACGAAGACTGCAATT  
GTTCTATCTATCCGGCATATAACGGGTATCGCATGGATGGGATATGATGA  
TGAACTGGTCCCTACGGCAGCGTTGGTAGCTCAGCTGCTCCGGATCCCA  
CAAGCCATCATGGACATGATCGTGGTCTACTGGGAGCTCTGGCGGGCAT  
AGCGTATTCATGGGGAACTGGCGAAGGTCTGGTAGTGTGCTGC  
TATTGCGCGTCGACCGGAAACCCACGTACCCGGGGAAAGTGGCGCC  
CACCAAGGCTGGCTTGTGGTCTCCTACACCAGGCGCCAAGCAGAACATCC  
AACTGATCAACACCAACGGCAGTGGCACATCAATAGCACGGCTTGA  
AATGAAAGCCTAACACCCGGCTGGTAGCAGGGCTTACCGATT  
AACTCTCAGGCTGCTGAGAGGTTGGCAGCTGCCAGCCCTTACCGATT  
GCCCAAGGGCTGGGGCTCTACAGTTATGCCAACCGAAGCGGCTCGACGAAC  
GCCCTACTGCTGGCACTACCCCTCAAGACCTTGTGGCATTGTGCGGGCAAAG  
AGCGTGTGGCCCGTATATTGCTTACTCCAGCCCCGGTGGGGAAAC  
GACCGACAGGTGGCGCCCTACCTACAGCTGGGCTGCAAATGATACGGAT  
GTCTTCGTCCTAACAAACACCAAGGCCACCGCTGGCAATTGGTGTG  
TGGATGAACTCAACTGGATTACCAAAAGTGTGCGAGCGCCCTTGTG  
CGGAGGGTGGCAACAAACACCTTGTCTGCCCCACTGATTGTTCCG  
ACCATATTCAAGTCAGGATGTACGTGGAGGGCTGAGACACAGGCTGG  
CGGCCTGCAACTGGACGGGGCGAACGGCTGTGATCTGGAAAGACAGGG  
GTCCGAGCTAGCCATTGCTGTGTCACCACACAGTGGCAGGTCTCC  
GTTCTTCACGACCCCTGCCAGCCTGTCCACCGGCTCATCCACCTCC  
ACATTGTGGACGTGCAGTACTTGTACGGGGTAGGGTCAAGCATCG  
GCCATTAAAGTGGGAGTACGTGTTCTCCTGTTCTGCTGAGAC  
GTCTGCTCTGTTGTGATGATGTTACTCATATCCAAGCGGAGGG  
GAGAACCTCGTAATACTCAATGCAGCATCCCTGGCGGGACGCACGG  
GTGTTGT

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GTCCCTCCTCGTCTTCTGCTTGCGTGGTATCTGAAGGGTAGGTGGGTGCC  
CGGAGCGGTCTACGCCCTACTACGGGAAGTGGGTCTTACTCTTATACCACATCTT  
AGTGGTACACCCAATCAAATCTGTAATTGTGATCCTACTGATGATTGGGATGT  
GGTAAAGGCCGATTCAAGGGGCCAAGAGTACTTGGGAAAATAGACCTCTGTT  
TTACAACAGTAGTACTAACGTCATAGGTTAACATAGCTAGGCCTGACCCAA  
CTATAGGCCACTGGTAACAATAATGGCAGCACTGAGGGTCACTGAACGTACC  
CACCAAGCCTGGAGTTGACATCGCTGTGGCGGTCACTGACTATAACCCACTGAT  
GGTAGCTATGTGACAGATTATTTAGATATAAAAATGGTTACAGTGCATTCTC  
AGCCTGGTATCTGCGGTGTTCTTGATAAGAAGCCTAATATACTTAGGTAGAATC  
GAGATGCCAGAGGTAACTATCCCCTAACTGGAGACCCTAACCTTAATACTATTA  
TATTGATCTCAACACAAATTGTAACGAGGTGGAAAGGTTGACGTGGCTGGCTA  
TTGTTGCAATGTGCGCTATCTTATTGCTGGTCACAACCTTGTGGGCCACTTCT  
TAACCCCTAATAACTGATCCTGCCTACCTATGAATTGGTTAAATTATACTATCTGAA  
AACTGTTAGGACTGATACAGAAAGAAGTTGGCTAGGGGGATAGACTATACAA  
GAGTTGACTCCATCTACGACGTTGATGAGAGTGGAGAGGGCGTATATCTTTTC  
CATCAAGGCAGAAAGCACAGGGAAATTCTATACTCTTGCCCCCTATCAAAG  
CAACACTGATAAGTTGCGTCAGCAGTAAATGGCAGCTAATATACTGAGTTACT  
TAACCTTGGACTTTATGTAACATGCACAGGAAAGTTAGAAGAGATCTCAG  
GAGGTACCAACATAATATCCAGGTTAGTGGCAGCACTCATAGAGCTGAACCTGG  
TCCATGGAAAGAAGAGGAGAGCAAAGGCTAAAGAAGTTTATCTATGTCCTGG  
AAGGTTGAGAAACCTAATAATAAAACATAAGGTAAAGGAATGAGACCGTGGCTT  
CTTGGTACGGGGAGGGAGGAAGTCTACGGTATGCCAAAGATCATGACTATAATC  
AAGGCCAGTACACTGAGTAAGAGCAGGCACTGCATAATATGCACTGTATGTGA  
GGGCCGAGAGTGGAAAGGTGGCACCTGCCAAAAATGTGGACGCCATGGAAAG  
CCGATAACGTGGGATGTCGTAGCAGATTGAAAGAAAGACACTATAAAAG  
AATCTTATAAGGGAAAGGCAACTTTGAGGGTATGTGCAAGCCGATGCCAGGGAA  
AGCATAGGAGGTTGAAATGGACCGGAAACCTAACAGTGGCAGATACTGTGCT  
GAGTGTAAATAGGCTGCATCCTGCTGAGGAAGGTGACTTTGGCAGAGTCGAG  
CATGTTGGCCTCAAAATCACCTACTTTGCGCTGATGGATGGAAAGGTGTATGA  
TATCACAGAGTGGCTGGATGCCAGCGTGTGGGAAATCTCCCCAGATACCCACA  
GAGTCCCTGTACATCTCACATTGGTTACGGATGCCCTTCAGGCAGGAATACA  
ATGGCTTGTACAATATACCGCTAGGGGCAACTATTCTGAGAAACCTGCCCG  
TACTGGCAACTAAAGTAAAAATGCTCATGGTAGGCAACCTTGGAGAAAGAAATT  
GGTAATCTGGAACATCTGGGTGGATCCTAAGGGGGCTGCCGTGTGTAAGAA  
GATCACAGAGCACGAAAATGCCACATTAATATACTGGATAAAACTAACCGCATT  
TTTGGGATCATGCCAAGGGGACTACACCCAGAGCCCCGGTGGAGGTTCCCTA  
CGAGCTTACTAAAGTGGAGGGCTGGAGACTGCCTGGCTTACACACAC  
CAAGGCCGGATAAGTCAGTCGACCATGTAACCGCCGGAAAAGATCTACTGGT  
CTGTGACAGCATGGGACGAACTAGAGTGGTTGCCAAAGCAACACAGGTTGA  
CCGATGAGACAGAGTATGGCGTCAGACTCAGGGTGGCCAGACGGTGC  
CAGATGTTATGTGTTAAATCCAGAGGCCGTTAACATATCAGGATCCAAAGGGG  
CAGTCGTTACCCCTCCAAAAGACAGGTGGAGAATTACGTTGTCACCGCATTCA  
GGCACACCGGGCTTCTCGACCTAAAAAACTGAAAGGATGGTCAGGCTTGCCT  
ATATTGAAAGCCTCCAGCGGGAGGGTGGTGGCAGAGTCAAAGTAGGGAAAGA  
ATGAAGAGTCTAAACCTACAAAATAATGAGTGGAAATCCAGACCGTCTCAAAAAA  
ACAGAGCAGACCTGACCGAGATGGTCAAGAAGATAACCGCATGAACAGGGG  
AGACTTCAAGCAGATTACTTGGCAACAGGGCAGGCAAAACACAGAACACTCC  
CAAAGCAGTTATAGAGGAGATAGGAAGACACAAAGAGAGTATTAGTTCTTATA  
CCATTAAGGGCAGCGGCAAGACTCAGTCTACCAAGTATATGAGATTGAAACACCC  
AAGCATCTCTTAACTTAACCTAACGGTAGGGGACATGAAAGAGGGGACATGGCAA  
CCGGGATAACCTATGCATCACGGGTACTTCTGCCAAATGCCCAACCAAAGC  
TCAGAGCTGCTATGGTAGAATACTCATACATATTCTAGATGAATACCATTGTGC  
CACTCCTGAACAACTGGCAATTATCGGGAAAGATCCACAGATTTCAGAGAGTAT  
AAGGGTTGTCGCCATGACTGCCACGCCAGCAGGGTCGGTGACCACAAACAGGT

FIGURE 24-2

CAAAAGCACCCAATAGAGGAATTCATAGCCCCCGAGGTAAATGAAAGGGGAGG  
ATCTTGGTAGTCAGTTCCITGATATAGCAGGGTTAAAATACCAAGTGGATGAGA  
TGAAAGGCCAATATGTTGGTTTGATACCAACGAGAAACATGGCAGTAGAGGTA  
GCAAAGAACGCTAAAGCTAAGGGCTATAACTCTGGATACTATTACAGTGGAGA  
GGATCCAGCCAATCTGAGAGTTGTGACATACAATCCCCATGTAATCGTGGC  
TACAATGCTATTGAATCAGGAGTGACACTACCAGATTGGACACGGTTATAGA  
CACGGGGTTGAAATGTGAAAAGAGGGTGGAGGTATCATCAAAGATAACCTTC  
TCGTAACAGGCCTAAGAGGATGGCGTGACTGTGGGTGAGCAGGCGCAGCG  
TAGGGGCAGAGTAGGTAGAGTGAAACCCGGGAGGTATTATAGGAGCCAGGAA  
ACAGCAACAGGGTCAAAGGACTACCAACTATGACCTCTGCAGGCACAAAGATA  
CGGGATTGAGGATGGAATCAACGTGACGAAATCCTTAGGGAGATGAATTACG  
ATTGGAGCCTATACGAGGAGGACAGCCTACTAATAACCCAGCTGGAAATACTA  
ATAATCTACTCATCTCAGAACAGACTGCCAGCCGCTGTTAGAACATAATGCC  
AGGACTGATCACCCAGAGCCAATCCAACCTGCATACAAACAGCTATGAAGTCCA  
GGTCCCAGGTCTGTTCCAAAATAAGGAATGGAGAAGTCACAGACACCTACG  
AAAATTACTCGTTCTAAATGCCAGAAAGTTAGGGAGGATGTGCCCCGTGATA  
TCTACGCTACTGAAGATGAGGATCTGCAGTTGACCTCTTAGGGCTAGACTGG  
CCTGATCTGGGAACCAGCAGGTAGTGGAGACTGGTAAAGCACTGAAGCAAGT  
GACCGGGTTGTCCTCGGCTGAAAATGCCCTACTAGTGGCTTATTGGGTATGT  
GGGTTACCAAGGCTCTCTAAAGAGGCATGTCCTAACATGATAACAGACATATAC  
CATCGAGGACCAGAGACTAGAACAGACACCACCCACCTCCAGTATGCACCCAACG  
CCATAAAAACCGATGGGACAGAGACTGAACAGACTGAAGAACACTGGCGTGGGTGA  
CGTGGAAAAAAATCATGGGAGGCCATTTCAGATTATGCAGCTGGGGACTGGAGT  
TGTGAAATCCCAAGCAGAAAAGATAAAAACAGCTCTTGTGTTAAAGAAAACG  
CAGAAGCCGCAAAGGGTATGTCCAAAATTCACTGACTCATTAATTGAAAATA  
AAGAAGAAAATAATCAGATATGGTTGTGGGAACACACAGCACTATACAAA  
AGCATAGCTGCAAGACTGGGCATGAAACAGCGTTGCCACACTAGTGTAAA  
GTGGCTAGCTTGGAGGGGAATCAGTGTCAAGACCACGTCAAGCAGCGGCA  
GTTGATTAGTGGTCTATTATGTGATGAATAAGCCTCTTCCCAGGTGACTCC  
GAGACACAGCAAGAAGGGAGGCGATTCTGCGCAAGCCTGTTATCTCCGCACT  
GGCAACCTACACATACAAAACCTGGAATTACCAATCTCTAAAGGGTGGGA  
ACCAGCCCTGGCTTACCTCCCTATGCTACAGCGCATTAAAATGTTACCC  
AACCGCGCTGGAGAGCGTGGTGAATCTGAGCACCACGATATAAAACATACC  
TCTCTATAAGGAAGGGGAAGAGTGTGATGGATTGCTGGTACGGGATAAGTGC  
AGCCATGGAAATCTGTACAAAACCCAGTATCGGTAGGTATATCTGTGATGTT  
GGGGGTAGGGGCAATCGCTGCGACAACGCTATTGAGTCCAGTGAACAGAAA  
AGGACCTACTATGAAGGTGTTGTAAGAAACTCTGGATCAGGCTGCAACA  
GATGAGCTGGTAAAAGAAAACCCAGAAAAAAATTATAATGGCTTATTGAAGCA  
GTCCAGACAATTGGTAACCCCTGAGACTAAATATACCACCTGTATGGGTTAC  
TACAAAGGGTGGAGGCCAAGGAACATCTGAGAGGAGCAGCAGGAGAAACT  
TATTCACTTGTGATAATGTTGAAGCCTCGAGTTAGGGATGGACTACAAG  
GGAAAATAAGGAACCTGCGGAAATTACATTGGATTGATATACGGCTAC  
ACAAGCAAATCACAGAGGGCTGAAGAAAATGGTACTGGGTGGGCCCTGC  
ACCCCTTACTGACTGGACCCCTAGTGACGAGAGGATCAGATTGCCAACAG  
ACAACATTGAGGGTGAAGAACCCAGGTGCCATGTTGCTATGAGATGAAAGCT  
TTCAAAATGTAGGTGGCAAACCTACCAAAAGTGGAGGAGAGCGGGCTTCT  
ATGTAGAAACAGACCTGGTAGGGGACCGAGTCAACTACAGAGTCACCAAGTATT  
ACGATGACAACCTCAGAGAGATAAAACCAAGTAGCAAAGTGGAGGACAGGTA  
GAGCACTACAAAGGGTCAAGCAGAAAAATTGACTACAGTAAAGGAAAAT  
GCTCTGGCCACTGACAAGTGGGAGGTGAAACATGGTGTATAACCAAGGTAG  
CTAAGAGATATACTGGGTGGCTCAATGGTGCATACCTAGGTGACGAGGCC  
AATCACCGTGTCTAGTGGAGAGGGACTGTGCAACTATAACCAAAACACAGT  
ACAGTTCTAAAATGAAGAAGGGTGTGCGTTACCTATGACCTGACCATCTC  
CAATCTGACCAGGCTCATCGAACTAGTACACAGGAACAATCTGAAGAGAAGG

FIGURE 24-3

AAATACCCACCGTACGGTCACCACATGGCTAGCTTACACCTCGTGAATGAAG  
ACGTAGGGACTATAAAACCACTAGTACTAGGAGAGAGAGTAATCCCCGACCCCTGTA  
GTTGATATCAATTACAACCAGAGGTGCAAGTGGACACGTCAGAGGTTGGGAT  
CACAATAATTGGAAGGGAAACCTGATGACAACGGGAGTGACACCTGCTTGG  
AAAAAGTAGAGCCTGACGCCAGCGACAACCAAACCTCGGTGAAGATCGGGTTG  
GATGAGGGTAATTACCCAGGGCCTGGAATACAGACACATAACACTAACAGAAGA  
AATACACAAACAGGGATGCGAGGCCCTCATCATGATCCTGGCTCAAGGAATT  
CCATATCAAATAGGGAAAGACTGCTAGAAATATAATCTGTACACAGGAAATG  
ACCCCAGGGAAATACGAGACTGATGGCTGCAGGGCGCATGTTAGTAGCA  
CTGAGGGATGTCGACCCCTGAGCTGCTGAAATGGTCATTCAAGGGACTTT  
TTAGATAGGGAGGGCCCTGGAGGCTCAAGTCTCGGGCAACCTAAACCGAAGC  
AGGTTACAAGGAAGCTGTTAGGAATTGATAGAACAGAAAAAGATGTGGAG  
ATCCCTAACTGGTTGATCAGATGACCCAGTATTTCTGGAAGTGGCTTAAAAA  
AATGATAAGTACTACTAGTAGGAGATGTTGGAGAGGTAAAAGATCAAGCTAA  
AGCACITGGGCCACGGATCAGACAAGAAATTATAAAGGAGGTAGGCTCAAGG  
ACGTATGCCATGAAGCTATCTAGCTGTTCTCAAGGCATCAAACAAACAGATG  
AGTTAACCTCACTGTTGAGGAATTGTCAGGTTGCACTGCAACTAAAG  
AGCAATAAGGGCACATGGCATCAGCTTACCAATTGGCACAGGGTAACTGGGA  
GCCCTCGGTTGCGGGGTGACCTAGGTACAATACCAGCCAGAAGGGTGAAG  
ATACACCCATATGAAGCTTACCTGAAGTTGAAAGATTCTAGAAGAAGAAGAG  
AAGAACCTAGGGTTAAGGATACAGTAATAAGAGAGCACAACAAATGGATACT  
TAAAAAAATAAGGTTCAAGGAAACCTCAACACCAAGAAAATGCTCAACCTGG  
GAAACTATCTGAACAGTTGGACAGGGAGGGCGCAAGAGGAACATCTACAAC  
CACCAGATTGGTACTATAATGTCAGTGCAGGCATAAGGCTGGAGAAATTGCC  
AATAGTGAGGGCCAAACCGACACCAAAACCTTCATGAGGCATAAGAGATA  
AGATAGACAAGAGTGAAAACCGGAAAATCCAGAATTGCAACAACAAATTGTTG  
GAGATTTCACACGATAGCCAACCCACCTGAAACACACACTACGGTGAGGT  
GACGTGGGAGCAACTTGAGGCGGGATAAAATAGAAAGGGGGCAGCAGGCTTC  
CTGGAGAAGAACATCGGAGAAGTATTGGATTGAGAAAAGCACCTGGTAGA  
ACAATTGGTCAGGGATCTGAAGGCCGGAGAAAGATAAAATATTGAAACTG  
CAATACCAAAATGAGAAGAGAGATGTCAGTGTACTGGCAGGCAGGGGA  
CTGGTGGTTGAGAAGAGGCCAAGAGTTATCCAATACCTGAAGCCAAGACAA  
GGCTAGCCATCACTAAGGCATGTATAACTGGGTGAAACAGCAGCCGTTGTG  
ATTCCAGGATATGAAGGAAAGACCCCTGTTCAACATCTTGATAAAGTGAGA  
AAGGAATGGACTCGTTCAATGAGGCCAGTGGCCGTAAAGTTTGACACCAAAGC  
CTGGGACACTCAAGTACTAGTAAGGATCTGCAACCTATTGGAGAAATCCAGA  
AATATTACTATAAGAAGGAGTGGCACAAGTTCATGACACCATCACCGACCACA  
TGACAGAAGTACCACTTATAACAGCAGATGGTGAAGTATATAAGAAATGGG  
CAGAGAGGGAGCGGCCAGCCAGACACAAGTGTGGCAACAGCATGTTAAATG  
TCCTGACAATGATGTACGCCCTCTGCAAGAACAGCACAGGGTACCGTACAAGAGT  
TTCAACACGGTGGCAAGGATCCACGTGTGGGATGATGGCTTCTTAATAAC  
TGAAAAAAGGGTTAGGGCTGAAATTGCTAACAAAGGGATGAGATTCTTCATG  
AAGCAGGCAAACCTCAGAAGATAACGGAAGGGAAAAGATGAAAGTTGCCTAT  
AGATTGAGGATATAGAGTTCTGTTCTCATACCCAGTCCCTGTTAGGTGGTCC  
GACAACACCACTAGTCACATGGCCGGAGAGACACCGCTGTGATACTATCAA  
GATGGCAACAAGATTGGATTCAAGTGGAGAGAGGGTACCCAGCATATGAAA  
AAGCGGTAGCCTTCAGTTCTGCTGATGTTACCTGGAAACCCGCTTGTAGGA  
GGATTGCGCTGTTGGCTTCTGCAACAGCCAGAGACAGACCCATCAAACATG  
CCACTTATTATTACAAAGGTGATCCAATAGGGCCTATAAAAGATGTAATAGGT  
GGAATCTAAGTGAACGTAAAGAGAACAGGGTTGAGAAATTGGCAAATCTAAAC  
CTAAGCCTGTCCACGTTGGGATCTGGACTAAGCACACAAGCAAAAGAATAAT  
TCAGGACTGTGTGCCATTGGGAAAGAAGAGGGCAACTGGCTAGTTAACGCCG  
ACAGGCTGATATCCAGCAAACCTGGCCACTTATACATACCTGATAAAGGTTA  
CATTACAAGGAAAGCATTATGAGCCAATGAGCTAAGAACAGAGACAAACCG

FIGURE 24-4

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GTCATGGGGTTGGGACTGAGAGATAACAAGTTAGGTCCCCATAGTCAATCTGCT  
GCTGAGAAGGTTGAAAATTCTGCTCATGACGGCCGTGGCGTCAGCAGCTGAG  
acaaaaatgtataatgtaaataaattaatccatgtacAATTCCGCCCTCTCCCTCCCCCCCCCTAACG  
TTACTGGCCGAAGCCGCTTGGAAATAAGGCCGTGCGTTGTCTATATGTTAT  
TTTCCACCATATTGCCGTCTTGGAAATGTGAGGGCCGAAACCTGGCCCTG  
TCTTCTTGACGAGCATTCTAGGGTCTTCCCTCTGCCAAAGGAATGCAAG  
GTCTGTTGAATGTCGTGAAGGAAGCAGTCCCTCTGGAAGCTTCTGAAGACAAA  
CAAACGTCTGTAGCGACCCTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGG  
TGCCTCTGCCGCAAAAGCCACGTGTATAAGATAACACCTGCAAAGGCCGACA  
ACCCCAGTGCACGTTGTAGTTGGATAGTTGTGAAAGAGTCAAATGGCTCT  
CCTCAAGCGTATTCAACAAGGGCTGAAGGATGCCAGAAGGTACCCATTGT  
ATGGGATCTGATCTGGGGCTCGGTGCACATGCTTACATGTGTTAGTCGAG  
GTTAAAAAAACGCTAGGCCACAAACatgaccgagtacaagcccacggtgccgcacccgcac  
AACACGATGATAAGCTTGCACAAACatgaccgagtacaagcccacggtgccgcacccgcac  
cgtccccccggccgtacgcacccctcgccggcgactacccgcacccgcac  
gagcgggtacccgagctgcaagaactttccctacgcgcgtcgccgtcgcac  
cggcggccgtctggaccacgcggagacgcgtgaagcggggccgtgtcgcc  
cggtcccccggctggccgcgcagcaacagaatggcgtccgtggccgcacc  
cgtcggcgtctggccgcaccaccaggcaagggctggcagcgcgtcgcc  
gggtgcccgccttctggagacctcgcgcggcccaaccttccctac  
gcccgaaggaccgcgcgaccctggtgcatgaccgcgaccggccgtcgcc  
aaaggagcgcacgaccccatgaaATGCATCGTACGAATTACGCCCACAGGCTGATAT  
CCAGCAAAACTGGCCACTTATACATACCTGATAAAGGCTTACATTACAAGGAA  
AGCATTATGAGCAACTGCAGCTAAGAACAGAGACAAACCCGGTATGGGGTT  
GGGACTGAGAGATAACAAGTTAGTCCCATAGTCAATCTGCTGCTGAGAAGGTT  
GAAAATTCTGCTCATGACGGCCGTGGCGTCAGCAGCTGAGacaaaaatgtataatgtaaata  
aattaatccatgtataatataatgtttggaccgtccacctaagaagacgac  
tcaagattatctaccctaagataacactacatgtatgcacacagcac  
ttagctgtatgaggatacgcccgacgtctatagtggactagg  
gaagacccctaaacagcccc

FIGURE 24-5

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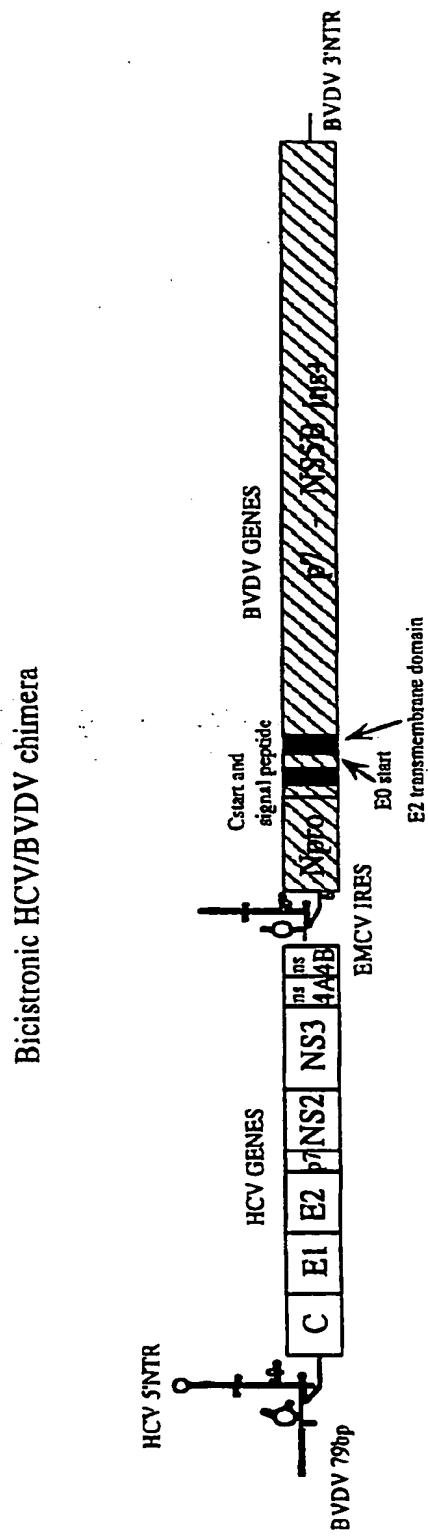


FIGURE 25

Gtatacgagaattagaaaaggcactcgatacgatgttggcaattaaaaataataattaggcctaggtacatggcacgtgccagccccct  
gatgggggcacactccaccatgaatcaactccctgtgaggaactactgtctcacgcagaaggcgttagccatggcgttagtatgag  
tgtcgccagtcctccaggaccggggccctccggagagccatagtggctcggaacccgtgagtagacaccggaaatggcaggacgac  
cgggtcccttcttgataaaccgcataatgcctggagatggcgcccccgaagactgttagccgagtagtgggtcgcaaa  
ggcccttgttactgcctgatagggtcgagtgcccgagggtcgtagaccgtgaccATGAGCACGAATC  
CTAAACCTCAAAGAAAAACCAAACGTAAACACCAACCCTGCCACAGGACGTC  
AAGTTCCC GG GTGGCGT CAG AT CGT TGG AG TT ACT TT GCG CG CAG  
GGGCCTAGATTGGGTGTGCGCGACGAGGAAGACTTCCGAGCGGTGCGCAA  
CCTCGAGGTAGACGTCAGCTATCCCCAAGGCACGTGCGCCCGAGGGCAGGA  
CCTGGGCTCAGCCC GG GT ACC CT TGG CCCC T AT GG CA AT GAGGTTGCGGG  
TGGGCGGGATGGCTCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGGGCCCCAC  
AGACCCCCGGCGTAGGTGCGCAATTGGTAAGGTCACTGATACCCCTACGT  
GCGGCTTCGCCAACCTCATGGGGTACATACCGCTCGTCCGGCCCCCTTGG  
GGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAAGACGGCGTGA  
ACTATGCAACAGGAACCTCTGGTGTCTTCTATCTTCTTCTGGCCCT  
GCTCTTGTGCTGACCGTGCCGCTTCAGCCTACCAAGTGCAGCAATTCTCGGG  
GCTTACCATGTCACCAATGATTGCCCTAACCTCGAGATTGTGTACGAGGCGGC  
CGATGCCATCTGCAACACTCCGGGGTGTGCCCCCTTGCCTCGGAGGGTAACG  
CCTCGAGGTGTGGGTGGCGGTGACCCCCACGGTGGCCACCAAGGGACGGCAA  
ACTCCCCACAACGCAAGCTCGACGTCAATACGATCTGCTTGTGGAGCGCCA  
CCCTCTGCTCGGCCCTCTACGTGGGGACCTGTGCGGGTCTGTCTTCTGGT  
GTCAACTGTTACCTTCTCTCCAGGCGCACTGGACGACGCAAGACTGCAATT  
GTTCTATCTATCCC GG CCATATAACGGGTCACTCGATGGCATGGATATGATGA  
TGAACTGGTCCCCCTACGGCAGCGTTGGTAGCTCAGCTGCTCCGGATCCA  
CAAGCCATCATGGACATGATCGTGGTCTACTGGGAGTCTGGGGCAT  
AGCGTATTCTCATGGTGGGAACTGGCGAAGGTCTGGTAGTGTGCTGCTGC  
TATTTGCCGGCGTCGACCGGGAAACCCACGTACCCGGGAAAGTGCCGGCCG  
CACCA CGG CTGG CT GTGG CT CCCC T AC ACCAGG CG CC AAGCAGAACATCC  
AACTGATCAACACCAACGGCAGTTGGCACATCAATAGCACGGCTTGAAC TG  
AATGAAAGCCTTAACACCGGCTGGTAGCAGGGCTCTCTATCAGCACAAATT  
AACTCTCAGGGTGTCTGAGAGGGTGGCCAGCTGCCAGCCCTACCGATT  
GCCCAAGGGCTGGGTCTTACAGTTATGCCAACGGAAAGCGGCCCTGACGAAC  
GCCCTACTGCTGGCACTACCCTCAAGACCTTGTGGCATTGTGCCCCGCAAAG  
AGCGTGTGTGGCCGGTATATTGCTTCACTCCCAGCCCCGGTGGTGGGAAC  
GACCGACAGGTGGCGCCTACCTACAGCTGGGGTGCACATGATACGGAT  
GTCTTGTCTTAACACACCAGGCCACCGCTGGCAATTGGTCTGGTTGTACC  
TGGATGAACTCAACTGGATTACCAAAAGTGTGCGGAGCGCCCCCTGTGTCAT  
CGGAGGGGTGGCAACAAACACCTTGCTCTGCCCAACTGATTGTTCCGCAAGC  
ATCCGGAAGCCACATACTCTCGGTGCTGCTCCGGTCCCTGATTACACCCAGG  
TGCATGGTCGACTACCCCTATAGGCTTGTGCACTATCTTGTACCATCAATTAC  
ACCATATTCAAAGTCAGGATGTACGTGGAGGGTCGAGCACAGGCTGGAAG  
CGGCCTGCAACTGGACCGGGCGAACGCTGTGATCTGGAAAGACAGGGACAG  
GTCCGAGCTCAGCCCATTGCTGCTGTCACCACACAGTGGCAGGTCTTCCGT  
GTTCTTCAAGGACCCCTGCCAGCCCTGTCCACCGGCTCATCCACCTCCACCA  
ACATTGTGGACGTGCACTTGTACGGGGTAGGGTCAAGCATCGCGTCTTGG  
GCCATTAAAGTGGGAGTACGTCGTTCTCTGTTCTCTGCTTGCAGACGCGCGC  
GTCTGCTCCTGCTTGTGGATGATGTTACTCATATCCAAGCGGAGGCGGGCTT  
GAGAACCTCGTAATACTCAATGCAGCATCCCTGGCGGGACGCACGGTCTTGT  
GTCCTCCTCGTGTCTGCTTGTGGTATCTGAAGGGTAGGTGGGTGCC

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CGGAGCGGTCTACGCCTCTACGGGA GTTGGCCTCTCCCTCTGCTCCTGCTGG  
 CGTTGCCTCAGCGGGCATACGCACTGGACACGGAGGTGGCCGCGTCGTGTTGG  
 CGGCCTTGTCTTGTGGGTTAATGGCGCTGACTCTGTCGCCATTACAAGCG  
 CTACATCAGCTGGTCATGTGGTGGCTTCAGTATTTCTGACCAGAGTAGAACG  
 GCAACTGCACGTGTGGGCCCCCTAACGTCCGGGGGGGGCGCGATGCC  
 GTCATCTTACTCATGTGTGTTGACACCCGACTCTGGTATTGACATCACCAAAC  
 TACTCCTGGCACTTCGGACCCCTTGGATTCTCAAGCCAGTTGCTAAAGT  
 CCCCTACTCGTGCCTCAAGGCCTCTCCGGATCTGCGCGTAGCGCGGA  
 AGATAGCCGGAGGTATTACGTCAAATGGCCATCATCAAGTTAGGGCGCTT  
 ACTGGCACCTATGTGATAACCATCTCACCCCTTTCGAGACTGGGCGACAAC  
 GGCTCGAGATCTGGCGTGGAACCAGTCGTCTCTCCCGAATGGA  
 GACCAAGCTCATCACGTGGGGGCAGATACCGCCCGTAGCGGTGACATCATC  
 AACGGCTTGCCTCTCGCCGTAGGGGCCAGGAGATACTGTTGGGCCAGC  
 CGACGGAATGGTCTCCAAGGGTGGAGGTTGCTGGCGCCATCACGGCGTAC  
 GCCCAGCAGACGAGAGGGCTCTAGGGTGTATAATCACCAGCCTGACTGGCG  
 GGACAAAAAACCAAGTGGAGGGTGGAGGTCAAGATCGTCAACTGCTACCCAAA  
 CCTTCCTGGCAACGTGCATCAATGGGTATGCTGGACTGTCTACCAACGGGCC  
 GGAACGAGGACCATCGCATCACCCAAGGGCTCTGTCATCCAGATGTATACCAA  
 TGTGGACCAAGACCTTGTGGGCTGGCGCTCTCAAGGTTCCGCTATTGA  
 CACCCCTGCACCTGCGGCTCCTCGGACCTTACCTGGTACCGAGGACGCCGAT  
 GTCACTCCGTGCCTCGGAGGTGATAGCAGGGTAGCCTGCTTCGCCCCG  
 GCCCATTCCTACTTGAAGGCTCTCGGGGGTCCGCTGTTGTGCCCCGCGG  
 GACACGCCGTGGGCTATTCAAGGCCCGGTGTGCACCCCTGGACTGGCTAA  
 GGCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAAACCATGAGAATCCCCGG  
 TGTTCACGGACAACCTCCTCCACCGCAGCGTAAGAGCACCAAGGTCCCCTGCGTA  
 CGCAGCCCAGGGCTACAAGGTGTTGGTGTCAACCCCTCTGTTGCTGCAACGC  
 TGGGCTTGGTGTACATGTCCAAGGCCATGGGGTGTACCTAATATCAGGA  
 CCGGGGTGAGAACAAATTACCAACTGGCAGCCCCATACGTACTCCACCTACGGC  
 AAGTTCTTGCAGGGGGGTGCTCAGGAGGTGTTATGACATAATAATTGT  
 GACGAGTGCCACTCCACGGATGCCACATCCATCTGGGATCGGCACTGTCT  
 TGACCAAGCAGAGACTGCCGGGGGAGACTGGTTGTGCTCGCCACTGCTACC  
 CCTCCGGGCTCCGTCACTGTGTCCTACCTAACATCGAGGAGGTGCTCTGTC  
 ACCACCGGAGAGATCCCCCTTACGGCAAGGCTATCCCCCTCGAGGTGATCAA  
 GGGGGGAAGACATCTCATCTCTGCCACTCAAAGAAGAAGTGCAGAGCTCG  
 CCGCGAAGCTGGTCGATTGGCATCAATGCCGTGGCTACTACCGCGGTCTT  
 GACGTGTCTGTCATCCCGACCAGCGCGATGTTGCGTGTGACCGATGC  
 TCTCATGACTGGCTTACCGCGACTTCGACTCTGTGATAGACTGCAACACGTG  
 TGTCACTCAGACAGTCGATTTCAGCCTGACCCCTACCTTACCATGAGACAAAC  
 CACGCTCCCCCAGGGATGCTGTCCAGGACTAACGCCGGGGCAGGACTGGC  
 AGGGGGGAAGCCAGGCATCTACGAGTTGTGGCACCAGGGGAGCGCCCCCTCG  
 GCATGTGCACTCGTCCGCTCTGTGAGTGTGATAGCTGACGCCGGCTGTGCTTGG  
 TATGAGCTCACGCCGCCAGAGACTACAGTTAGGCTACGAGCGTACATGAACAC  
 CCCGGGGCTTCCCGTGTGCCAGGACCATCTGAATTGGGAGGGCGTCTTAA  
 CCGGGCTCACTCATATAGATGCCCACTTCTATCCAGACAAAGCAGAGTGGG  
 GAGAACTTCTTACCTGGTACGCTACCAAGCCACCGTGTGCGCTAGGGCTCA  
 AGCCCCCTCCCCCATCGTGGGACCAAGATGTGGAAGTGTGATCCGCCTTAAAC  
 CCACCCCTCCATGGCCAACACCCCTGCTATACAGACTGGGCGTGTCAAGAAT  
 GAAGTCACCCCTGACGCACCCAACTACCAAATACATCATGACATGCACTGCGCC  
 GACCTGGAGGTGTCACGAGCACCTGGGTGCTCGTTGGCGCGTCTGGCTG  
 CTCTGGCCCGTATTGCCGTCAACAGGCTGCGTGGTCAAGTGGGAGGGATT  
 GTCTTGTCCGGGAAGCCGGCAATTATACCTGACAGGGAGGGTCTCTACCAAGGA  
 GTTCGATGAGATGGAAGAGAGTGTCTCAGCACTTACCGTACATCGAGCAAGGGA  
 TGATGCTCGCTGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCTGAGACCCCG

FIGURE 26-2

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TCCCGCCAAGCAGAGGTTATCACCCCTGCTGTCCAGACCAACTGGCAGAAACT  
 CGAGGTCTCTGGCGAAGCACATGTGGAATTCTCATCAGTGGATACAATACTT  
 GGCAGGCGCTGTCAACGCTGCCGTGTAACCCCCCATTGCTTCATTGATGGCTTT  
 TACAGCTGCCGTACCAGCCACTAACACTGGCAAACCCCTCCCTTCACAT  
 ATTGGGGGGTGGGTGGCTGCCAGCTGCCGCCCCGGTGCGCTACCGCC  
 TTGTGGCGCTGGCTTAGCTGGCAGGGTATGGCGGGCGTGGCGGGAGCTCT  
 AGGTCTCGTGACATTCTGCAGGGTATGGCGGGCGTGGCGGGAGCTCA  
 TGAGCCTCAAGATCATGAGCGGTAGGTCCTCCACGGAGGACCTGGTCA  
 ATCTGCTGCCGCCATCCTCTGCCCTGGAGCCCTGTAGTCGGTGTGGCTGC  
 GCAGCAATACTGCCGGCACGTTGGCCGGAGGGGGAGTGAATGGA  
 TGAACCGGCTAATAGCCTCGCCTCCGGGAAACCATGTTCCCCCACGCAC  
 TACGTGCCGGAGAGCGATGCAGCCGCCGCTCACTGCCATACTCAGCAGCCT  
 CACTGTAACCCAGCTCTGATcgCTAGccatgggtaccgagCGTTACTGGCCAGGCC  
 GCTTGAATAAGGCCGGTGTGCGTTGTCTATGTTATTTCACCATATTGCC  
 GTCTTGGCAATGTGAGGGCCGGAAACCTGGCCCTGTCTTGACGAGCA  
 TTCTAGGGTCTTCCCCTCGCCAAGGAATGCAAGGTCTGTTGAATGTCG  
 TGAAGGAAGCAGTCCCTCTGGAAGCTTCTTGAAAGACAAACACGTCTGTAGCG  
 ACCCTTGCAAGCAGCGGAACCCCCCACCTGGCAGCAGGTGCCTCTGCCAG  
 AAAGCCACGTGTATAAGATACACCTGCAAAAGGGCGACAACCCAGTGCACG  
 TTGTGAGTGGATAGTGTGAAAGAGTCAAATGGCTCTCAAGCGTATTCA  
 ACAAGGGCTGAAGGATGCCAGAAGGTACCCATTGATGGGATCTGATCTG  
 GGGCCTCGGTGACATGCTTACATGTTAGTCGAGGTTAAAAAACGTCTAG  
 GCCCCCGAACACGGGGACGTGGTTTCCTTGAAAAAACACGATGATAATAT  
 GGAGTTGATCACAAATGAACCTTATACAAACATACAAACAAAACCGTCGG  
 GGTGGAGGAACCTGTTATGATCAGGCAGGTGATCCCTATTGGTGAAGGG  
 GAGCAGTCCACCCCAATCGACGCTAACAGCTCCACACAAGAGAGGGGAACGC  
 GATGTTCCAACCAACTTGCATCCTACCAAAAGAGGTGACTGCAGGTGG  
 TAATAGCAGAGGACTGTGAGCGGGATCTACCTGAAGCCAGGGCACTATT  
 ACCAGGACTATAAAGTCCGTCTATCACAGGGCCCGTGGAGCTCTTGAG  
 GAGGGATCCATGTGAAACGACTAACCGATAGGGAGAGTAACGGAAAGTG  
 ACGGAAAGCTGTACCAACTTATGTGTATAGATGGATGATAATAATAAAA  
 GTGCCACGAGAAGTACCAAGGGTGTCAAGGTGGGCTATAATAGGCTTGAC  
 TGCCCTCATGGTCACAAGTGTCAAGCACGAAAGAGAGGGAGCAACAAAG  
 ctGCATTGTTGGCGTGGCAATAATAGCTATAGTTGTTCAAGTTACAATGGG  
 AGAAAACATAACACAGTGGAACccgagTGGTTGACCTGGAGGTGACTGACCAT  
 CACCGGGATTACTCGCTGAGTCCATATTAGTGGTGTAGTAGCCCTTGGG  
 GGCAGATATGTACTTGGTACTGGTACATGATGGGACTGGCAACTTGCT  
 GCCTTAGGGATTCACTGGATCAGGGGAAGTGGTGTAGTGGCAACTTGCT  
 AACCCATAACAATATTGAAGTGGTACATACTCTTGCTGTACCTACTGCT  
 GAGGGAGGAGAGCGTAAGAAGTGGCTTACTCTTACACACATCTTGTGG  
 TACACCAATCAAATCTGTAATTGTGATCCACTGATGATTGGGATGTGGTAA  
 AGGCCGATTCAAGGGGGCAAGAGTACTGGGAAAATAGACCTCTGTTTACA  
 ACAGTAGTACTAATCGTCATAGGTTAATCATAGCTAGGCGTGAACCAACTATA  
 GTGCCACTGGTAACAATAATGGCAGCACTGAGGGTCACTGAACCTGACCCACCA  
 GCCTGGAGGTGACATCGCTGGCGGTCACTGACTATAACCCACTGATGGTTA  
 GCTATGTGACAGATTATTAGATATAAAAATGGTACAGTGCATTCTCAGCCT  
 GGTATCTGGGTGTTCTGATAAGAAGCCTAATATACCTAGGTAGAATCGAGAT  
 GCCAGAGGTAACATCCAAACTGGAGACCAACTACTTAAACTATTATATTG  
 ATCTCAACAACAATTGTAACGAGGTGGAGGGTACGTGGCTGGCGACTCTTAAAC  
 GCAATGTGTGCTATCTTATTGCTGGTCACAACCTGTGGCGACTCTTAAAC  
 CCTAATACTGATCCTGCTACCTATGAATTGGTTAAATTATACTATCTGAAA  
 GTTAGGACTGATATAGAAAGAAGTGGCTAGGGGGAGAGACTATACAAGAGT  
 TGACTCCATCTACGACGGTGTGATGAGAGTGGAGAGGGCGTATATCTTCCATC  
 AAGGCAGAAAGCACAGGGAAATTCTATACTCTGCCCCCTATCAAAGCAAC

FIGURE 26-3

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ACTGATAAGTTGCGTCAGCAGTAAATGGCAGCTAATATACATGAGITACTTAAC  
TTGGACTTTATGTACTACATGCACAGGAAAGTTAGAAGAGAGATCTCAGGAGG  
TACCAACATAATATCCAGGTAGTGGCAGCACTCATAGAGCTGAACCTGGTCCAT  
GGAAGAAAGAGGAGGAGCAAAGGCTAAAGAAGTTTATCTATTGTCTGGAAAGGT  
TGAGAAACCTAATAATAAAACATAAGGTAAAGGAATGAGACCGTGGCTTCTGGT  
ACGGGGAGGAGGAAGTCTACGGTAGGCCAAAGATCATGACTATAATCAAGGCC  
AGTACACTGAGTAAGAGCAGGCACTGCATAATATGCACTGTATGTGAGGGCCG  
AGAGTGGAAAGTGGCACCTGCCAAAATGTGGACGCCATGGGAAGGCCATA  
ACGTGTGGATGTCGCTAGCAGATTGAAAGAAAGACACTATAAAAGAATCTTT  
ATAAGGGAAAGGCAACTTGAGGGTATGTGAGCCATGCCAGGGAAAGCATA  
GGAGGTTGAAATGGACCGGAACCTAACAGAGTGCCAGATACTGTGCTGAGTGT  
AATAGGCTGCATCCTGCTGAGGAAGGTGACTTTGGCAGAGTCGAGCATGTT  
GGGCCTCAAAATCACCTACTTGCCTGATGGATGGAAAGGTGTATGATATCAC  
AGAGTGGGCTGGATGCCAGCGTGTGGGAATCTCCCCAGATACCCACAGAGTCC  
CTTGTACATCTCATTTGGTCACGGATGCCCTTCAGGCAGGAATAACATGGCT  
TTGTACAATATACCGCTAGGGGCAACTATTCAGGAAACTTGGCCCTACTGG  
CAACTAAAGTAAAAATGCTCATGGTAGGCACCTGGAGAAGAAATTGGTAATC  
TGGAACATCTGGGTGGATCCTAACGGGGGCTGCCGTGTGAAGAACATCAC  
GAGCACGAAAATGCCACATTAAATAATACTGGATAAAACTAACCGCATTTCGGG  
ATCATGCCAAGGGGACTAACCCAGAGCCCCGGTAGGGTCCCACACACACCAAGGC  
ACTAAAAGTGGAGGGGCTGGAGACTGGCTGGCTTACACACACACACACAC  
GGGATAAGTTCACTGACCATGTAACCGCCGGAAAAGATCTACTGGCTGTGA  
CAGCATGGGACGAACTAGAGTGGTTGCCAAAGCAACAAACAGGTGACCGATG  
AGACAGAGTATGGCTCAAGACTGACTCAGGGTCCCCAGACGGTGCCAGATG  
TTATGTGTTAAATCCAGAGGCCGTTAACATATCAGGATCCAAGGGCAGTCG  
TCACCTCCAAAAGACAGGTTGAGAATTACGTGTCACCGCATCAGGCACAC  
CGGCTTCTCGACCTAAAAACTGAAAGGATGGTCAGGCTTGCCTATATTG  
AAGCCTCCAGCGGGAGGGTGGTGGCAGAGTCAAAGTAGGAAAGAACAG  
GTCTAACCTACAAAAATAATGAGTGGAAATCCAGACCGTCTCAAAAAACACAGC  
AGACCTGACCGAGATGGTCAAGAACAGATAACCAGCATGAACACAGGGAGACTTCA  
AGCAGATTACTTGGCAACAGGGCAGGCAAAACACACAGAACACTCCAAAAGCA  
GTTATAGAGGAGATAGGAAGACACAAGAGAGTATTAGTTCTATACCATTAAAGG  
GCAGCGGCAGAGTCAGTACCAAGTATATGAGATTGAAACACCAAGCATCTC  
TTTAACTTAAGGATAGGGGACATGAAAGAGGGGACATGGCAACCGGGATA  
ACCTATGCATCATACGGTACTTCTGCCAAATGCCCTAACCAAAGCTCAGAGCT  
GCTATGGTAAATACTCATACATATTCTAGATGAATACCATTGTGCCACTCCTG  
AACAACTGGCAATTATCGGAAGATCCACAGATTTCAGAGAGTATAAGGGTT  
GTCGCCATGACTGCCACGCCAGCAGGGTGGTAGCCACAAACAGGTCAAAAGC  
ACCCAATAGAGGAATTACAGCCCCGAGGTAAATGAAAGGGGAGGATCTGGT  
AGTCAGTTCTTGTATAGCAGGGTAAAAATACCAAGTGGATGAGATGAAAGG  
CAATATGTTGGTTTTGTACCAACGGAGAAACATGGCAGTAGAGGTAGCAAAGA  
AGCTAAAAGCTAAGGGCTATAACTCTGGATACATATTACAGTGGAGAGGATCCA  
GCCAATCTGAGAGTTGTGACATCACAATCCCCATGTAATGTGGCTACAAAT  
GCTATTGAATCAGGAGTGACACTACCAGATTGGACACGGTTAGAACACCGGG  
GTTGAAATGTGAAAAGAGGGTGGGGTATCATCAAAGATACCCCTCATCGTAA  
CAGGCCTTAAGAGGATGCCGTGACTGTGGGTGAGCAGGGCAGCGTAGGG  
CAGAGTAGGTAGAGTGAAACCCGGGAGGTATTATAGGAGCCAGGAAACAGCA  
ACAGGGTCAAAGGACTACCACTATGACCTTGCAGGCACAAAGATACGGGAT  
TGAGGATGGAATCAACGTGACGAAATCCTTAGGGAGATGAATTACGATTGGA  
GCCATACGAGGAGGACAGCCTACTAATAACCCAGCTGGAAACTAAATAATC  
TACTCATCTCAGAAGAGACTGCCAGCGCTGTTAGAACATAATGGCCAGGACTG  
ATCACCCAGAGCCAATCCAACCTGCATACAAACAGCTATGAAGTCCAGGTCCC  
GTCCTGTTCCAAAATAAGGAATGGAGAAGTCACAGACACCTACGAAAATTAC  
TCGTTCTAAATGCCAGAAAGTTAGGGAGGATGTGCCGTGTATCTACGCT

FIGURE 26-4

ACTGAAGATGAGGATCTGGCAGTTGACCTCTTAGGGCTAGACTGGCCTGATCC  
 TGGGAACCAGCAGTAGTGGAGACTGGTAAAGCACTGAAGCAAGTGACCGGG  
 TTGTCCCTCGGCTGAAAATGCCCTACTAGTGGCTTATTGGGTATGTGGGTTAC  
 CAGGCTCTCTCAAAGAGGCATGTCCCAATGATAACAGACATATACCATCGAG  
 GACCAGAGACTAGAAGACACCACCCACCTCCAGTATGCACCCAACGCCATAAA  
 AACCGATGGACAGAGACTGAAGAAACTGGCGTGGGTGACGTGGAA  
 AAAATCATGGGAGGCCATTTCAGATTATGCAGCTGGGGACTGGAGTTGTTAA  
 ATCCCAGCAGAAAAGATAAAAACAGCTCTTGTAAAGAAAACGCAGAAGC  
 CGCAAAAGGGTATGTCCAAAATTCAATTGACTCATTAAATTGAAAATAAGAAGA  
 AATAATCAGATATGGTTTGTGGGGACACACACAGCACTATACAAAAGCATAGC  
 TGCAAGACTGGGCATGAAACAGCTTGCACACTAGTGTAAAGTGGCTAG  
 CTTTGGAGGGAAATCAGTGTAGACACCACGTCAAGCAGGCGCAGTTGATTTA  
 GTGGCTATTATGTGATGAAATAAGCCTTCCCTCCAGGTGACTCCGAGACACAG  
 CAAGAAGGGAGGCATTGTCGCAAGCCTGTTCATCTCGCACTGGCAACCTA  
 CACATACAAAACITGGAATTACCACAATCTCTAAAGTGGTGAACCGAGCCCT  
 GGCTTACCTCCCCATGCTACCAGCGCATTAAAAATGTTACCCCCAACGGCGCT  
 GGAGAGCGTGGTGTAACTGAGCACACGATATATAAAAACATACTCTTATAAG  
 GAAGGGGAAGAGTGTGGATTGCTGGGTACGGGGATAAGTGCAGCCATGGAA  
 ATCCTGTACAAAACCCAGTATCGTAGGTATATCTGTGATGTTGGGGTAGG  
 GGCAATCGCTGCGCACACGCTATTGAGTCAGTGAACAGAAAAGGACCCCTAC  
 TTATGAAGGTGTTGTAAGAAACTCTTGGATCAGGCTGCAACAGATGAGCTGG  
 TAAAAGAAAACCCAGAAAAAATTATAATGGCCTTATTGAAGCAGTCCAGACAA  
 TTGGTAACCCCCCTGAGACTAATATACCACCTGTATGGGTTACTACAAAGGTT  
 GGGAGGCCAAGGAACATCTGAGAGGACAGCAGGAGAAAATTATTACATTGAC  
 ATAATGTTGAAGCCTTCAGTTATTAGGGATGGACTCACAGGGAAAATAAG  
 GAACCTGTCCGAAATTACATTGGATTTGATATAACGGCTACACAAGCAAAT  
 CAACAGAGGGCTGAAGAAAATGGTACTGGGGTGGGCCCTGACCCCTTGTAGTT  
 GTGACTGGACCCCTAGTGACGAGAGGATCAGATTGCCAACAGACAATTG  
 AGGGTAGAAAACCCAGGTGCCCATGTGGCTATGAGATGAAAGCTTCAAAAATGT  
 AGGTGGCAAACCTACCAAAGTGGAGGAGAGCGGGCTTCTATGTAGAAACA  
 GACCTGGTAGGGGACCAGTCAACTACAGAGTCACCAAGTATTACGATGACAAC  
 CTCAGAGAGATAAAACCACTAGCAAAGTTGGAAGGACAGGTAGAGCACTACTA  
 CAAAGGGGTACAGCAAAATTGACTACAGTAAAGGAAAATGCTTGGCCA  
 CTGACAAGTGGAGGTGAAACATGGTGTATAACCAGGTTAGCTAAGAGATAT  
 ACTGGGGTCGGGTTCAATGGTGCATACTTAGGTGACGAGGCCAACACCGTGC  
 TCTAGTGGAGAGGGACTGTGCAACTATAACCAAAACACAGTACAGTTCTAAA  
 AATGAAGAAGGGTGTGCGTTCACCTATGACCTGACCACCTCAATCTGACCA  
 GGCTCATCGAACTAGTACACAGGAACAATTGAAAGAGAAGGAAAATACCCACC  
 GCTACGGTCACACATGGCTAGCTTACACCTTCTGTGAATGAAGACGTAGGGAC  
 TATAAAACCACTAGGAGAGAGTAATCCCCGACCCCTGTAGTTGATATCAA  
 TTACAACCAAGAGGTGCAAGTGGACACGTAGAGGTTGGGATCACATAATTG  
 GAAGGGAAACCTGATGACAACAGGGAGTGAACACCTGTCTGGAAAAAGTAGA  
 GCCTGACGCCAGCGACAACAAAACCTCGGTGAAGATCGGGTTGGATGAGGGT  
 AATTACCCAGGGCCTGGAATACAGACACATACACTAACAGAAGAAAATACCAA  
 CAGGGATGCGAGGGCCCTCATCATGATCTGGCTCAAGGAATTCCATATCAA  
 ATAGGGCAAAGACTGCTAGAAAATATAATCTGTACACAGGAATGACCCAGG  
 GAAATACGAGAGACTTGTGATGGCTGCAGGGCGATGTTAGTAGACTGAGGGA  
 TGTCACCCCTGAGCTGTGAAATGGTGTGTTCAAGGGACTTTTAGATAG  
 GGAGGCCCTGGAGGCTTAAGTCTCGGGCAACCTAACCGAAGCAGGTTACCA  
 AGGAAGCTGTTAGGAATTGATAGAACAGAAAAAGATGTGGAGATCCCTAAC  
 TGGTTGATCAGATGACCCAGTATTCTGGAAAGTGGCCTTAAAAAATGATAAG  
 TACTACTAGTAGGAGATGTTGGAGAGGTAAAAGATCAAGCTAAAGCAGTGG  
 GCCACGGATCAGACAAGAATTATAAAGGAGGTAGGCTCAAGGACGTATGCCA  
 TGAAGCTATCTAGCTGGTTCTCAAGGCATCAAACAAACAGATGAGTTACTC

FIGURE 26-5

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CACTGTTGAGGAATTGTTGCTACGGTGCCACCTGCAACTAAGAGCAATAAG  
GGGCACATGGCATCAGCTTACCAATTGGCACAGGGTAACTGGGAGGCCCTCGG  
TTGGGGGTGCACCTAGGTACAATACCAGCCAGAAGGGTGAAGATAACCCAT  
ATGAAGCTTACCTGAAGTTGAAAGATTTCATAGAAGAAGAGAAGAAACCT  
AGGGTTAAGGATACAGTAATAAGAGAGCACAACAAATGGATACCTAAAAAAAT  
AAGGTTCAAGGAAACCTCAACACCAAGAAAATGCTAACCCCTGGGAAACTATC  
TGAACAGTGGACAGGGAGGGCGCAAGAGGAACATCTACAACCACAGATT  
GGTACTATAATGTCAAGTGCAGGCATAAGGCTGGAGAAATTGCCAATAGTGAG  
GCCCAAACCGACACCAAAACCTTTCATGAGGCAATAAGAGATAAGATAGACA  
AGAGTAAAACCGGCAAAATCCAGAATTGACACAACAAATTGTTGGAGATTTCC  
ACACGATAGCCCACCCACCCCTGAAACACACCTACGGTGAGGTGACGTGGGAG  
CAACTTGAGGCAGGGATAAAATAGAAAGGGGGCAGCAGGCTCTGGAGAAGA  
AGAACATCGGAGAAGTATTGGATTCAAGAAAAGCACCTGGTAGAACAAATTGGTC  
AGGGATCTGAAGGCCGGGAGAAAGATAAAATATTATGAAACTGCAATACCAAA  
AAATGAGAAGAGAGATGTCAGTGACTGGCAGGCAGGGACCTGGTGGTT  
GAGAAGAGGCCAAGAGTTATCCAATACCCCTGAAAGCCAAGACAAGGCTAGCCAT  
CACTAAGGTATGTATAACTGGGTGAAACAGCAGGCCGTTGTGATTCAGGAT  
ATGAAGGAAAGACCCCTTGTCAACATCTTGATAAAAGTGAGAAAGGAATGG  
GACTCGTTCAATGAGCCAGTGGCGTAAGTTTGACACCAAAGCCTGGACAC  
TCAAGTGACTAGTAAGGATCTGCAACTTATTGGAGAAATCCAGAAATTTACTA  
TAAGAAGGAGTGGCACAAGTTCATGACACCACATACCGACCATGACAGAAG  
TACCAAGTTATAACAGCAGATGGTGAAGTATATAAGAAAATGGCAGAGAGGG  
AGCGGCCAGCCAGACACAAGTGTGGCAACAGCATGTTAAATGTCTGACAAT  
GATGTACGCCCTCTGCGAAAGCACAGGGTACCGTACAAGAGTTCAACAGGG  
TGGCAAGGATCCACGCTGTGGGATGATGGCTTCAATAACTGAAAAAGGG  
TTAGGGCTGAAATTGCTAACAAAGGGATGAAAGTTGCCTATAGATTGAGG  
ACCTCAGAAGATAACGGAAGGGAAAAGATGAAAGTTGCCTATAGATTGAGG  
ATATAGAGTTCTGCTCATACCCCACTGCTTGTAGGTGGCCGACAACACCA  
GTAGTCACATGGCCGGGAGAGACACCGCTGTGATACTATCAAAGATGGCAACA  
AGATTGGATTCAAGTGGAGAGAGGGTACACAGCATATGAAAAAGCGGTAG  
CCTCAGTTCTGCTGATGTATTCTGGAACCCGCTTGTAGGTGGGTTAGGAGGATTGCT  
GTTGGCTCTTCGCAACAGCCAGAGACAGACCCATCAAACATGCCACTTATTA  
TTACAAAGGTATCCAATAGGGCCTATAAGATGTAATAGGTGGAAATCTAA  
GTGAAGTGAAGAGAACAGGCTTGTGAGAAATTGGCAAATCTAAACCTAAGCCTG  
TCCACGTGGGATCTGGACTAACGACACAAGCAAAAGAATAATTCAAGGACTG  
TGTGCCCCATTGGGAAAGAAGAGGGCAACTGGTAGTTAACGCCAGGGCTGA  
TATCCAGCAAAACTGCCACTTATACATACCTGATAAAGGCTTACATTACAAG  
GAAAGCATTATGAGCAACTGCAGCTAAGAACAGAGACAAACCCGGTCATGGGG  
GTTGGGACTGAGAGATAAACAGTTAGGTCCCAGTCAATCTGCTGCTGAGAAG  
GTTGAAAATTCTGCTCATGACGGCCGTCGGCGTCAGCAGCTGAgacaaaatgttatattgt  
aaataaaatccatgtacatagtgttatataatatagtgtggaccgtccacctcaagaagacgacacgcacagctaaac  
agtagtcaagattatctaccctcaagataacactacatttatgcacacagcacttagctgttatgaggatagccgacgttatagtg  
taggaaagaccctcaacagcccc

FIGURE 26-6

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/08850

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/29, 39/295; C12Q 1/70; C12N 7/01; C07H 21/02  
US CL: 424/218.1, 228.1; 435/5, 235.1; 536/23.72

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/218.1, 228.1; 435/5, 235.1; 536/23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
APS; Derwent/WEST; DIALOG

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| X,P       | FROLOV et al. cis-acting RNA elements required for replication of bovine viral diarrhea virus-hepatitis C virus 5' nontranslated region chimeras. RNA. November 1998, Vol. 4, pages 1418-1435, see entire document.   | 1-8, 10-21            |
| Y,P       | MALET et al. Yellow fever 5' noncoding region as a potential element to improve hepatitis C virus production through modification of translational control. Biochem. Biophys. Res. Commun. 18 December 1998, Vol. 253, No. 2, pages 257-264, see entire document. | 1-8, 10-21            |

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents:

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- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\*

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\*

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\*

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*&\*

document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

10 SEP 1999

19 JULY 1999

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/08850

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| Y         | LU et al. Poliovirus chimeras replicating under the translational control of genetic elements of hepatitis C virus reveal unusual properties of the internal ribosomal entry site of hepatitis C virus. Proc. Natl. Acad. Sci. USA. 20 February 1996, Vol. 93, No. 4, pages 1412-1417, see entire document. | 1-8, 10-21            |
| Y         | VASSILEV et al. Authentic and chimeric full-length genomic cDNA clones of bovine viral diarrhea virus that yield infectious transcripts. J. Virol. January 1997, Vol. 71, No. 1, pages 471-478, see entire document.  | 1-8, 10-21            |
| Y         | VENUGOPAL et al. Towards a new generation of flavivirus vaccines. Vaccines. 1994, Vol. 12, No. 11, pages 966-975, see entire document.  | 1-8, 10-21            |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/08850

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2.  Claims Nos.: 9 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
**CLAIM 9 RECITES "SEQ ID NO:X" WHICH EXPRESSION IS NOT UNDERSTOOD.**
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| (30) Priority Data:                                     | 60/082,964 24 April 1998 (24.04.98) US | (71) Applicant (for all designated States except US): | WASHINGTON UNIVERSITY [US/US]; One Brookings Drive, St. Louis, MO 63130 (US).  |
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(54) Title: CHIMERAS OF HEPATITIS C VIRUS AND BOVINE VIRAL DIARRHEA VIRUS

## (57) Abstract

Disclosed is a polynucleotide comprising a chimeric viral RNA which contains: a 5' nontranslated region (5' NTR), an open reading frame (ORF) region, and a 3' nontranslated region (3' NTR) wherein at least one of said regions is chimeric. The chimeric region comprises a first nucleotide sequence from a pestivirus in operable linkage with a heterologous nucleotide sequence. The chimeric viral RNA is replication-competent. Preferably the pestivirus sequence is from a bovine viral diarrhea virus and the heterologous nucleotide sequence is from a hepatitis C virus. Also disclosed are a method for identifying compounds having antiviral activity against hepatitis C virus, a genetically-engineered chimeric RNA virus and a vaccine against bovine viral diarrhea virus.

## Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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